# PROCEEDINGS OF THE WORKSHOP ON CAPTIVE BROODSTOCKS FOR RECOVERY OF IMPERILED SALMONID POPULATIONS

**25 – 26 June 2002**

**Prepared by**
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T.A. Flagg (NMFS)

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C. Busack (WDFW)

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Parameterizing a Quantitative Genetic Model of Natural and Artificial Selection During Supplementation
M. Ford (NMFS)

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## 11: 40 – 12:00: Overall wrap-up of (Technical Sessions 1-4)

**12:00 – 1:00 Lunch**

## 1:00 – 4:00: Session 5: Policy Perspectives on Captive Broodstocks

Pete Hassemer (IDFG)

Herb Pollard (NMFS)

Pat Patillo (WDFW)

## PREFACE

Captive broodstocks are becoming an increasingly important component of species preservation and restoration of ESA-listed salmonid populations in major river systems. Captive broodstocks have been implemented, or are being planned, for numerous Pacific and Atlantic salmon stocks. These range from coho salmon in central California to steelhead on Vancouver Island B.C., and Atlantic salmon in the northeastern United States and southeastern Canada. It seems certain that the number of salmon stocks conferred captive broodstock protection will continue to grow in the face of increasing risks of extinction. Nonetheless, captive broodstock technology as a tool for conservation of salmonids is still in its initial stages of development.

The purpose of the two-day Workshop was to bring together: (i) research scientists, to report their latest research findings to further the success of captive broodstock programs, (ii) managers of existing captive broodstock programs, to describe their persistent and most critical problems to enable scientists to focus their research, and (iii) fisheries managers, to outline their current policy perspectives on captive broodstocks and speculate on their future role in population recovery. Invited Oral Presentations were thematic, leading to open discussion. Plenary themes included the history and challenges of captive broodstock programs, genetics, growth and survival in captivity, maturation and reproduction, reintroduction strategies, and policy perspectives. Poster Presentations provided supporting input and overviews of current captive broodstock programs, monitoring and evaluation efforts, and research topics.

This report of the Workshop is in three parts. The first contains the extended abstracts submitted by authors of Oral Presentations, and the second is a summary of the question and answer sessions following each talk. The last part presents the abstracts of the Poster Presentations.

The Workshop was sponsored by the National Marine Fisheries Service, the Idaho Department of Fish and Game, the Oregon Department of Fisheries and Wildlife, and the Pacific Aquaculture Caucus.

The Organization Committee for the Workshop was: Barry A. Berejikian, Thomas A. Flagg, Jeffrey J. Hard, Colin E. Nash, and Penny Swanson (National Marine Fisheries Service), Peter F. Hassemer (Idaho Department of Fish and Game), and Timothy Hoffnagle (Oregon Department of Fish and Wildlife).

## PART I. EXTENDED ABSTRACTS FROM ORAL PRESENTATIONS

### Opening Remarks: History of Captive Broodstock Programs in the Pacific Northwest and Future Challenges

Thomas A. Flagg

National Marine Fisheries Services, Northwest Fisheries Science Center, Resource Enhancement and Utilization Technologies Division,
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About a decade ago, a conference by the Royal Zoological Society on the captive breeding of endangered animals was opened on a very positive note regarding the successes technologies for maintaining endangered populations (Olney et al. 1994). It is appropriate to open this Workshop on Captive Broodstocks for Recovery of Imperiled Salmonid Populations on a similar positive note. Salmonid captive broodstock technology has quickly progressed from a sound but rather untested concept (Flagg and Mahnken 1995) to a current position of playing a strengthening role in conservation of our most endangered stocks in the Pacific Northwest.

Worldwide, hundreds of species are being reared in captivity (Gipps 1991, Olney et al. 1994, DeBlieu 1991). Most of these *ex situ* populations are maintained in zoos and aquaria. While, as mentioned above, these efforts have been instrumental in preventing extinction by maintaining the species in captivity, few have the luxury of producing enough animals to truly make an impact towards recovery. Salmonid reproductive characteristics provide the possibility of this luxury for our captive broodstock programs--the high fecundity of salmon and the network of hatchery systems provide the real possibility to both reduce short-term extinction risk and speed recovery. However, concerns abound regarding the risks and appropriate use of this amplification potential. Issues of concern include genetic integrity, health, and physiological and behavioral quality; these will be addressed during the Workshop with a goal of helping guide future management decisions for the use of salmonid captive broodstocks.

An overview of the history of captive broodstocks in the Pacific Northwest can be structured in two components: what was known about the potential of the technology prior to its implementation in the early 1990s as a component of US Endangered Species Act (ESA) activities for salmon, and subsequent success of application. From the mid 1970s, a number of captive broodstock approaches had been initiated for stocks in the Pacific Northwest. These were a mixture of conservation, commercial, and research endeavors. The commercial and husbandry potential seemed apparent (McAuley 1981, Mighell 1981, Flagg et al. 1995a). However, conservation potential was often hampered by reduced adult size and low egg viability (both often less than 50%) and survival to adult of often less that 10% (Harrell et al. 1987, Flagg et al. 1995b, Schiewe et al. 1997).

The ESA recognizes artificial propagation as a potential conservation tool (Hard et al. 1992). Initial research into the use of captive broodstocks for ESA-listed stocks in the Pacific Northwest focused on culture parameters necessary to ensure survival. Research led to development of specialized broodstock facilities at several sites in the Northwest (e.g., National Marine Fisheries Service's Manchester Research Station, Idaho Department of Fish and Game's Eagle Hatchery) where fish are reared in secure land-based tanks supplied with water processed to ensure quality (e.g., reduce pathogens). These husbandry advancements have been noted to provide survival to adult normally averaging above 50% and ranging to 90% (Schiewe et al. 1998, Flagg et al. 1998, Flagg and Mahnken 2000). Life history parameters such as adult size, maturation, reproductive behavior, and egg viability have shown improvement associated with advancements in culture techniques; however, most still remain compromised compared to wild fish (Schiewe et al. 1997; Flagg and Mahnken 2000; Berejikian et al. 1997, 2001a). A major focus of current captive broodstock research is determination of exogenous and endogenous factors that contribute to reductions in fitness of captive broodstocks and determination of culture methodologies to remediate deficiencies (Berejikian 2000, 2001b).

Captive broodstocks such as the NMFS/IDFG Redfish Lake sockeye salmon program have been positively associated with protection of some of the Pacific Northwest's most endangered salmonid resources (Flagg et al. 1995, 1998; Flagg and Mahnken 2000). Captive broodstocks have the apparent potential to maintain genetic diversity of a population and to provide meaningful demographic population amplification. Modern captive broodstock programs differ significantly from the pre1990s programs in that they tend to be well funded and well coordinated between federal, state, tribal, university, and private investigators. Current programs in the Pacific Northwest have been founded on the soundest principles of genetics and species biology, are scaled to carrying capacity of receiving habitats, and include strong research, monitoring, and evaluation components to help ensure success. Nonetheless, captive broodstocks alone will not recover a population. Captive broodstocks *de facto* do not address factors for decline associated with the majority of anadromous salmonid migratory life history. While captive broodstocks can provide the seeds for recovery, often far reaching management decisions affecting all sectors of anthropogenic impacts will be necessary to for meaningful recovery to proceed.

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Flagg, T.A., F.W. Waknitz, and C.V.W. Mahnken. 1995a. The captive broodstock concept: application to Pacific salmon. *In* T. A. Flagg and C. V. W. Mahnken (editors), An assessment of the status of captive broodstock technology for pacific salmon, pages 1-1 to 1-60.

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McAuley, W.C. 1981. DOMSEA coho broodstock program. *In* T. Nosho (editor), Salmonid broodstock maturation, p. 23-24. Proceedings of the salmonid broodstock maturation workshop. Univ. Washington Sea Grant Pub. WSG-WO 80-1.

Mighell, J.L. 1981. Culture of Atlantic salmon, *Salmo salar*, in Puget Sound. U.S. Natl. Mar. Fish. Serv. Mar. Fish. Rev. 43(2):1-8.

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## Balancing Genetic Risks and Demographic Benefits In Captive Broodstock Programs

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*EXTENDED ABSTRACT – DO NOT CITE*

The primary purpose of this talk is to discuss the genetic risks inherent in captive brood (CB) programs and to provide a context for this discussion by comparing these risks to the risks posed by "classic" supplementation (CS) programs. To do this properly, it is critical that the benefits of such programs be discussed as well. The uncertainty involved in assessing either risk or benefit is very large, making risk-benefit analysis much more of a concept than a real evaluation tool, and it is important that those implementing these programs understand this.

Any method that uses artificial propagation to increase natural production of a population is supplementation. Therefore, although CB methods are often compared to supplementation as if they were something else, CB methods, like CS methods are merely a subset of possible supplementation methods. Fundamentally, CB methods are subject to the same constraints, uncertainties, and risks as any other supplementation method.

The overriding constraint on supplementation programs is the limitations on NOR (natural-origin recruit) increase imposed by natural productivity and capacity. Supplementation alone is not the cure for population smallness unless smallness is the disease. Thus, a supplementation program implemented to counter a temporary precipitous drop in population size or to counter a depensatory survival situation may

actually solve the problem. In all other cases, however, the population can be expected to return to its presupplementation condition after supplementation is stopped. If genetic degradation has taken place as a result of the supplementation program, the population may be worse off than it was before supplementation was implemented. Understanding this constraint is key to assessing supplementation benefits

The overriding uncertainty about supplementation is whether it works. There are no known examples of a supplementation program, once discontinued, having restored a population to sustainability. This is methodologically not a particularly good measure of success, but supplementation requires at least that the program return enough adults of adequate spawning ability to increase the number of NORs. Few if any supplementation programs have enough information on reproductive success of hatchery fish to determine if this is happening, but studies of this sort are now underway. This demographic benefit also must be achieved while keeping genetic impacts below a debilitating level, and we are far from understanding these impacts.

Hatchery programs can subject populations to four types of genetic hazards: extinction, loss of within-population diversity, outbreeding depression/loss of among-population diversity, and domestication. It is impossible to generalize about the relative extinction risk posed by CB and CS. CB can be either more or less risky, depending on the situation. Loss of within population diversity, typically caused by decreasing effective size can be much greater in CB programs if only a few fish are represented in the breeding population, but CB programs also offer the possibility of keeping effective size higher than CS programs can because of the ability to manipulate the relative contributions of individuals. For risks caused by population mixing (outbreeding depression and loss of among-population diversity), the relative risk of the two types of programs is again situation specific. Collecting only the target stock may or may not be easier with CB. Risks posed to other populations from straying may be greater with CB if the release sizes are larger, but otherwise CB poses no additional risk unless the CB method makes fish more apt to stray. Domestication is the area of greatest concern in comparing CB with CS methods. To the extent that length of hatchery residence and difference between hatchery and natural environments cause domestication, CB methods seem to have more potential for inbreeding. It is hard to argue that rearing in captivity a fish that would normally go to sea and make extensive migrations before returning to spawn does not have substantial domestication potential. CB programs may also be riskier because they can put many more fish on the spawning grounds than CS programs. On the other hand, the additional risk may be less if CB programs are more short-lived than CS programs. Also in CB programs there is the potential for controlling family size, which can be used to reduce selection.

In general, the potential of CB programs to produce very large numbers of fish makes them appear riskier than CS programs, but this is countered by the potential they offer for controlling some sources of risk by population manipulation. There is also the possibility that even if a substantial genetic impact is incurred, the population can be grown to a size large enough that natural genetic processes can reduce or remove the impact over time without undue risk to the population.

**Inbreeding and its Consequences: What Do We Know and Need to Know?**

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*EXTENDED ABSTRACT – DO NOT CITE*

Inbreeding is a potent force for evolutionary change, but its dynamics in and consequences for salmonids are poorly understood. This information is missing while many salmon populations are in steep decline. Although these declines may arise from many factors, genetic variation provides the potential for adaptation and, therefore, avoidance of inbreeding and maintenance of genetic variability should be paramount considerations in conservation and management of salmonids. Attempts to conserve and recover imperiled populations must take genetic concerns into account to minimize problems that can occur during intervention.

Salmonids have received surprisingly little attention as a system to study inbreeding and its consequences. Most studies published to date have focused on the consequences of close inbreeding for growth and survival in captive freshwater species, especially rainbow trout. The dearth of work on these species, particularly those with anadromous life histories, is striking in the face of growing concerns about genetic effects of declining abundance and artificial propagation in these species. The lack of attention to salmonid inbreeding results in part from the longevity and complex life history of anadromous fish, which complicate and protract the requisite breeding studies.

Despite the paucity of work on inbreeding, some patterns are emerging from available studies. First, salmonids respond to inbreeding and exhibit inbreeding depression. Under an assumption of a linear relationship between inbreeding coefficient and phenotype, the reduction in phenotype with respect to fitness per 10% increase in inbreeding (for modest levels of inbreeding) ranges from about 3-15% under rapid inbreeding and 1-5% under slow inbreeding. The salmonid values are similar to those observed for other species. Inbreeding depression in these species therefore appears to depend on the rate of inbreeding. Second, the variability in estimates of inbreeding undoubtedly reflects diverse background and genetic histories of the strains evaluated in these studies. Third, although the relationship between inbreeding depression and coefficient of inbreeding may vary appreciably among species and traits, available evidence indicates that salmonid survival and growth during early life history can show responses to moderate levels of inbreeding.

Nevertheless, the mechanisms and consequences of inbreeding in salmonids remain elusive. Therefore, in 1994 we initiated a study of inbreeding and its consequences in Puget Sound fall chinook salmon to elucidate these processes. Although this study has not yet implemented a full generation of inbreeding, preliminary results indicate inbreeding depression in freshwater and early marine survival and growth can occur

within one generation of full-sib mating. However, the results are highly variable. Effects of inbreeding on developmental stability and survival to adulthood show some interesting preliminary patterns.

The population structure of anadromous salmon, when combined with frequently small population sizes and precise homing to natal streams, provides ample opportunity for inbreeding to occur. However, this opportunity can be magnified considerably under human intervention, and especially in captive broodstock programs. For example, matings among full- and half-siblings can occur rapidly in a small captive broodstock established from a relatively few number of founders unless care is taken to avoid them. To what extent or at what point this practice would reduce productivity in the broodstock and increase extinction risk for an associated natural population is not yet known.

Future research on the consequences of reduced genetic variability for salmon should focus on determining the traits most sensitive to inbreeding, comparing inbreeding depression in captive and hatchery populations, characterizing inbreeding depression over the entire life history, comparing responses to slow and rapid inbreeding, and evaluating selection as a means of purging to reduce adverse fitness consequences of inbreeding.

Until these issues are resolved, managers should limit opportunities for inbreeding. Breeding practices that maintain large effective broodstocks that are representative of the population remain important to minimize unwanted genetic change. Schemes that maximize genotypic combinations in the progeny each breeding season should be encouraged, and these practices should be directly coupled to regular genetic monitoring. Where feasible, pedigrees should be established to relate rates of inbreeding to different breeding patterns and to correlate these rates with measures of inbreeding depression.

**Infectious diseases in fresh and saltwater-reared salmon captive broodstock programs**

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*EXTENDED ABSTRACT – DO NOT CITE*

Columbia River Basin Captive Broodstock Programs are designed to save stocks of chinook and sockeye salmon whose existence have become endangered due to degraded freshwater habitat and/or impediments to natural migration. As with all fish culture, salmon reared in these programs can contract a variety of infectious diseases that result in considerable mortality. Under some circumstances losses due to infectious disease can be catastrophic. The causal agents of infectious diseases in salmon include a variety of parasitic, bacterial, and viral pathogens. There are many factors that contribute to the persistence and amplification of pathogens and the diseases they cause, including the lack or ineffectiveness of chemotherapeutics and vaccines, vertical transmission of many pathogens from females to their eggs, and the need to rear the fish in these programs for a long period of time, over a complete life cycle from eggs through spawning. In this talk, common and emerging infectious agents in salmon will be discussed in the context of their appearance in both fresh and saltwater captive broodstock programs. An overview of current research being carried out to develop improved treatments or vaccines for a number of these pathogens will also be presented.

**Parasitic diseases**

A number of parasitic diseases can and have caused morbidity and mortality in salmon captive broodstock. These include *Salmincola californiensis*, a copepod found in chinook salmon reared at the Eagle Fish Hatchery in Idaho, and the Rosette agent that in the past infected salmon reared in seawater at the NMFS Manchester Marine Laboratory and is currently occurring in the Sacramento winter-run chinook salmon program. As *Myxobolus cerebralis,* the causal agent of Whirling Disease, spreads westward in various trout populations, we need to remain aware of its possible impacts in captively reared anadromous salmonids.

**Bacterial pathogens**

The most devastating bacterial pathogen in Pacific salmon captive broodstock programs continues to be *Renibacterium salmoninarum*, the causal agent of Bacterial Kidney Disease or BKD. Long-lasting treatments and vaccines that eliminate the pathogen are still unavailable. Other important bacterial pathogens encountered in fresh water captive

broodstock include the motile Aeromonads (such as *A. hydrophila* and *A. sobria*), *Flavobacterium psychrophilum*, the causal agent of Cold Water Disease (CWD). *Flexibacter maritimus* is a marine flexibacterium that has been implicated in mortality in salt-water reared captive broodstock.

**Viral pathogens**

Losses due to viruses have been essentially non-existent in the captive broodstock programs until recently. All mortalities and spawning adults are carefully screened for the presence of the main salmonid viruses, including IHNV, VHSV, and IPNV. Infectious salmon anemia virus (ISAV) is not yet specifically included in these screens, but if this virus is ever detected in any Pacific salmon stocks, methodologies designed to specifically detect this virus will be included. A recent outbreak of IHNV occurred in captive Redfish Lake sockeye yearlings being reared at Bonneville Fish Hatchery, just prior to the time these fish would have been moved back to Idaho for release at Redfish Lake. After confirmation of the virus and as losses mounted (up to 3.5%/day), difficult management decisions had to be made regarding the disposition of these fish. A careful risk assessment by the Stanley Basin Sockeye Technical Oversight Committee indicated that the best course of action, one which NMFS eventually approved, was to destroy the stock to avoid exposure of other stocks to the virus, either in the Stanley basin or around the hatchery. The risk assessment matrix used by the parties involved should serve as a model for what action to take during any future epizootics of this or similar pathogen not endemic in the native habitat of the at-risk populations.

**Research in treatment and prevention strategies**

Some of the simplest treatment strategies involve rearing captive broodstock in pathogen-free water, preventing exposure to infected fish, vaccination, or treatment with various chemotherapeutics. However, in practice these strategies are not always easy to implement for a variety of reasons. For many pathogens such as *R. salmoninarum,* completely effective chemotherapeutics are not readily available and development of efficacious vaccines has proven to be challenging. Great strides have been made in the development of viral vaccines, including a DNA-based vaccine against IHNV. The Fish Health/Microbiology group at NWFSC/NMFS has been testing an alternative macrolide antibiotic azithromycin that may prove more effective than erythromycin in the treatment of salmon infected with this pathogen. In addition, tests using a commercial BKD vaccine called Renogen in combination with whole cells from an attenuated strain of *R. salmoninarum* shows promise as both a prophylactic and therapeutic agent against BKD.

The authors acknowledge the following for contributing information used in this talk: Rich Holt and Sam Onjukka (Oregon Department of Fish and Wildlife); Steve Roberts, Bob Rogers, and Keith Keown (Washington Department of Fish and Wildlife); Scott Foott and Kristen Arkush (Sacramento Winter Chinook Program); Garth Traxler and Dave Groves (British Columbia Department of Fisheries and Oceans); Carlin McAuley, and Cindy Rathbone (National Marine Fisheries Service); Eagle Lab staff (Idaho Department of Fish and Game). Funding has been provided by the Bonneville Power Administration.

**Repeated dietary administration of erythromycin impairs survival, growth, and fertility in chinook salmon (*Oncorhynchus tshawytscha*)**

**WilliamT. Fairgrieve[1], William C. McAuley[2], Cindy L. Masada[2], Mark E. Peterson[2], Mark S. Strom[2] and Thomas A. Flagg[2]**

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*EXTENDED ABSTRACT – DO NOT CITE*

## Introduction

Erythromycin has been used since the 1950s for preventing and treating bacterial kidney disease (BKD) in salmonids (Wolf and Dunbar 1959). It has been administered by injection to maturing adults, and used when water hardening eggs to reduce vertical transmission (Groman 1983) and as a feed additive for treating epizootics in presmolt salmonids. Prophylactic administration of erythromycin has become increasingly common, particularly in captive broodstock programs involving ESA listed stocks of sockeye and chinook salmon.

Studies with Lake Wenatchee sockeye salmon suggested that erythromycin has a negative effect on gamete viability. Eyed egg survival for sockeye prophylactically treated up to four times per year and spawned during 1994 and 1995 ranged from 40-60% (Flagg et al. 1997). Ovarian inflammation in rainbow trout fed erythromycin thiocyanate has been reported (Piper 1961). However, no experimental data are currently available regarding the effects of long-term erythromycin prophylaxis on gonad development and egg viability in any salmonid species.

Controlled studies to determine the effects of erythromycin on gamete quality are especially relevant to captive rearing of ESA listed stocks. Experiences with Catherine Creek, Lostine River, and Lemhi River spring chinook salmon have shown currently mandated treatment regimens may elicit fatal toxicity reactions. Affected fish exhibited reddened skin in the cranial region and swam erratically or convulsed before death, suggesting neurological involvement. Extensive liver damage has been observed. Experience has shown that terminating treatment alleviates the red-head condition. Similar toxic reactions have been reported in rainbow trout (Piper 1961), but no data currently exist for other salmonid species and it is not known if sensitivity increases with repeated treatments.

The objectives of this study with captive fall chinook salmon broodstock were to (1) determine how gonad development, gamete viability, and survival of their progeny though the swim-up stage is affected by long-term prophylactic administration of erythromycin and (2) if erythromycin toxicity responses are related to treatment

frequency, measure residual tissue concentrations, and document underlying tissue damage associated with the syndrome.

**Methods**

The experiment was conducted in two phases: the fry-to-smolt and the smolt-to-mature adult phases. In Phase 1, duplicate groups of fall chinook salmon (initial weight 0.42 g) were pair-fed diets medicated with erythromycin either once (ES-1), twice (ES-2), or three times (ES-3) over a period of 18 weeks, beginning two weeks after first-feeding (February 1999) and ending at smoltification (June 1999). Unmedicated diets were fed between treatments, and to the experimental control (E-0). Samples were collected to measure growth and whole body antibiotic concentrations. In Phase 2, fish from each Phase 1 treatment were PIT tagged and redistributed so a secondary treatment regimen could be applied. Duplicate groups of fish received either no additional treatment (E-0), or were fed diets medicated with erythromycin either twice (E-2) or four (E-4) times per year for two years.

Dietary drug concentrations and feeding rates were adjusted during each feeding period to deliver 100 mg (active) erythromycin/kg body weight per day for 28 days. Medicated feeds were prepared by spraying the antibiotic dissolved in ethanol (25 ml/kg feed) onto a commercial salmon diet. The fish were reared in pathogen-free well water (10°C) throughout the experiment.

Mature fish were spawned during August and September 2001. Females and males were paired in a factorial arrangement to permit detailed evaluation of male and female gamete quality. Fertilization and survival to the eyed stage was determined; fry were counted, measured, and weighed at swim-up; and samples taken for erythromycin analysis. Erythromycin residues in tissues were determined microbiologically.

**Preliminary results**

Medicated diets were well accepted by juvenile fall chinook salmon, and there were no differences in growth among the dietary treatments. Erythromycin was poorly absorbed by first feeding fry, but increased with fish size. Whole body concentrations averaged less than 0.2 ug/ml in first feeding fry and about 10 ug/ml at smoltification. Erythromycin was cleared from the tissues within 3 weeks post-treatment. No signs of toxicity were observed in any treatment group.

During the smolt-to-adult phase of the study, E-4 fish were highly sensitive to handling, exhibiting nervousness and elevated mortality. Overall survival was 93-96% in the E-0 and E-2 groups, compared with 71% in the E-4 treatment. Although fish in all treated groups readily accepted the medicated feed, growth in both treated groups was depressed dramatically. Mature fish averaged 1682 g, compared with 1587g and 1427 g for the E-2 and E-4 groups.

Preliminary analysis of spawning data suggests a trend toward reduced fecundity and survival to the eyed stage in E-4 groups, compared with the untreated control (E-0) and E-2 groups. These trends are apparent in all post-smolt treatment groups, without regard

to treatment applied during the presmolt period. These further review and statistical analysis of these data must be completed before final conclusions can be made.

Erythromycin residues were detected in about 21% of E-4 female spawners, compared with about 6% of those in the E-2 group. Carryover from some E-4 (but not E-2) females to the fry was also observed. Kidney tissue concentrations averaged 5.3 ug/ml in spawned females, and about 1.5 ug/ml in the tissues of the fry. *In vitro* bacteriostatic activity of erythromycin for *R. salmoninarum* is observed at concentrations of <0.67 to 21.87 ug/ml (Bandin et al. 1991).

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**Captive broodstock nutrition: what are we forgetting?**

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*EXTENDED ABSTRACT-DO NOT CITE*

Although there are broodstock diets available commercially there still is some question of the nutritional adequacy of the feed. Of interest is that analysis of the fish itself and the reproductive products have determined most of the nutrients thought to be required by broodstock. This analytical method is a good start but needs to be fined tuned. Not many well-structured feeding trials have been conducted to determine the fishes' nutritional requirements.

Some of the broodstock requirements are known. Vitamin C and E have been found to affect fecundity and fertilization. In addition to these vitamins other functions, they act as antioxidants to protect the essential highly unsaturated fatty acids (HUFAs) (Izquierdo et al. 2001). These HUFAs provide building blocks for successful spawn and reproductive performance. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are two HUFAs thought to be required for increased fecundity and hatching. Docosahexaenoic acid is also important for developing embryos. Arachidonic acid (AA) as well as EPA was found to have positive affects on fertilization rates. Table 1 has a fatty acid comparison of a prey item and a broodstock diet. The comparison illustrates the differences that can be found in a prey item and a commercial broodstock diet. Poston and Ketola (1989), in their comparison of composition of hatchery-raised and sea-run Atlantic salmon, found that prey items found in the stomach of the sea-run fish contained approximately 41.9% polyunsaturated fatty acids (includes HUFAs). The commercial brood diets contained 11.9-29.2% polyunsaturated fatty acids.

Vitamin levels in brood diets reportedly need to be at least double that of what juveniles require (Lee and Donaldson 2001). Table 2 shows the levels of select vitamins in a commercial brood diet. In juvenile Pacific salmon diets, vitamin A is required, vitamin E is required at 30 IU/kg diet and folic acid is required at 6-10 mg/kg diet. Clearly, research is needed in the area of broodstock vitamin nutrition to better meet the needs of fish. Some work is presently being done concerning the development of diets and feeding regimes for captively raised salmon (Berejikian 2000). Concerns being addressed are protein/energy as well as total food intake; total lipid in the fish; fatty acid and mineral composition of fish released from a captive rearing program (sockeye, Chinook); and the importance of carotenoids in the brood diet. In conclusion, there are still many aspects of broodstock nutrition that need investigation. It is important to have good nutrition for the health and survival of the adults and offspring.

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Table 1. Comparison of fatty acids in natural prey and a commercial broodstock diet

| Fatty acid (HUFA) | Prey item (krill) | Commercial broodstock diet |
|---|---|---|
| Arachidonic acid (AA) | 2.3* | 0.21 |
| Eicosapentaenoic acid (EPA) | 20.5 | 3.66 |
| Docosahexaenoic acid (DHA) | 12.8 | 2.62 |

* % of total fatty acids

Table 2. Vitamin levels in a commercial broodstock diet

| Vitamin | Commercial broodstock diet |
|---|---|
| A | 15,900 IU/kg |
| E | 385 IU/kg |
| Folic acid | 5.2 mg/kg |

## Growth regulation in captive broodstock rearing of salmon

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*EXTENDED ABSTRACT - DO NOT CITE*

The question of what is the best strategy for growing Pacific salmon in captive broodstock programs is not resolved. One strategy is to regulate growth rates of captive fish to match the wild stock's natural growth pattern and accordingly, life history phenotype, which would minimize loss of fitness and genetic selection in captivity. An alternate strategy would be to grow the fish as fast as practical to maximize production capacity and reduce rearing costs and risks. Either strategy has its own risks and benefits. This short review will discuss some of these issues and suggest where future research may be directed.

From the practical perspective, growth and development of fish are regulated to achieve critical sizes at specific times - size and date of ponding, target size for fish marking or tagging, smolt size for fish released or transferred to seawater, and adult reproductive criteria including age at maturation and fecundity. Fish size must be regulated to maintain appropriate densities in captivity to minimize stress and maintain good health. Growth rates are often dictated by water temperature and regulated by dietary ration and composition. In general, comparing the growth of salmon in captivity versus the wild, captive fish up to the smolt stage are larger than in the wild, whereas captive fish from the smolt to mature adult stages are smaller than in the wild. This general situation is dictated by the practical constraints of rearing in captivity. The early (freshwater) stages of salmon rearing in the wild are subject to extreme seasonal conditions. Winter conditions are often characterized by near-freezing temperature and prolonged periods of fasting. Summer conditions in the wild may include rearing temperatures (and opportunities for growth) that are well above the optimum for fish growth and disease resistance in captivity, e.g. temperature above 13º C (55º F). Nevertheless, optimal rearing strategies may be achieved to approximate natural growth cycles within the practical limitations of captive rearing.

Two major salmon life history events that are of concern in captive rearing of salmon are smoltification and reproductive maturation. The timing and quality of both of these developmental events are significantly influenced by modulation of growth rate. Control of smoltification is particularly important in captive rearing strategies that involve transfer of fish to seawater or release of fish at the smolt stage. Control of reproductive maturation is particularly important in captive brood programs to synchronize maturation of males and females or in captive rearing programs that release adults to synchronize maturation with wild spawners.

**Smoltification**

For some species, e.g., spring chinook salmon, the timing of smoltification is dependent on early growth rate (Beckman and Dickhoff, 1998). High rate of growth in the fry and parr stages promotes fall smolting, whereas slower growth rate will delay smolting until spring. Furthermore, high growth rate during smolting enhances smolt quality and downstream migration (Beckman et al., 1998, 1999). Simulating winter conditions in the wild by rearing fish at low temperature or prolonged fasting during winter may be used to limit smolt size with no apparent adverse effects on subsequent smolting in spring, at least in coho salmon (Larsen et al., 2001). For captive programs that employ smolt releases, the issue of smolt size at release is unresolved. To minimize genetic selection, one strategy would include releasing smolts at a size equivalent to wild smolts. However, releasing larger than natural smolts might provide a survival advantage (albeit perhaps an unnatural advantage), that would result in a greater smolt-to-adult survival (SAS). The potential loss of fitness resulting from release of exceptionally large smolts is unknown. Results from the Redfish Lake sockeye program showed that release of unnaturally large smolts significantly enhances SAS. The effects of this practice on the offspring of those large smolts is unknown.

**Reproductive maturation**

Experience to date indicates that the high early growth rate of salmon in captive broodstock (and supplementation) programs induces a high proportion of early maturing males (Shearer et al., 2000, 2001) and age of maturation, fecundity, and egg size, in female salmon (Campbell et al., 2001). There is clearly a problem with synchronizing maturation of males and females, since in some cases most of the males have matured before the females of the same year class. In contrast to the issue of size at smoltification, such variation in age of maturation would be expected to have significant genetic effects, not the least of which is effective population size ($N_e$). Critical to managing this problem is identifying the periods when the reproductive system is sensitive to the influence of growth rate. An initial study determined that early maturation of chinook salmon begins in the fall, one year prior to spawning (Shearer and Swanson, 2000). The current working hypothesis is that there are two decision periods (one in the fall and one in the spring), during which growth has a significant influence on maturation (Figure 1). If growth is high during both periods, then maturation goes to completion that year. If growth is low during the initial (fall) period, then high growth during the subsequent period in spring will not be sufficient to support maturation. Additional tests are needed to confirm this two-period decisional process. However, if it holds then it constitutes a rational strategy to control both maturation and smoltification of captive salmon.

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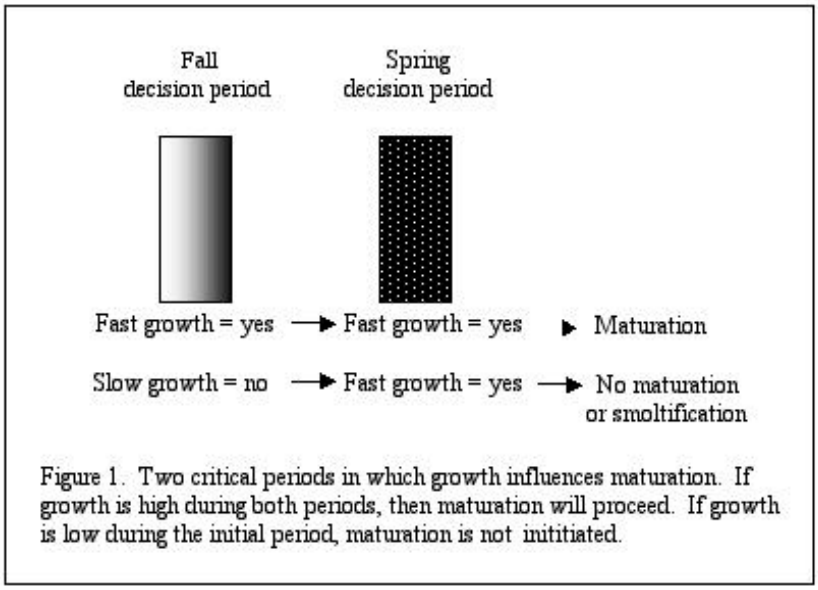


Figure 1. Two critical periods in which growth influences maturation. If growth is high during both periods, then maturation will proceed. If growth is low during the initial period, maturation is not inititiated.

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**Early sexual maturation in 1+ age male spring chinook salmon: examination of the roles of size and fatness**

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*EXTENDED ABSTRACT-DO NOT CITE*

In many salmonids, males may mature at an early age relative to females, with the incidence varying among species, stocks, and rearing conditions for cultured fish. Although early maturing males (precocious parr, jacks or mini-jacks) are a natural phenotype for Pacific salmon such as chinook salmon, it is undesirable to produce abnormal proportions of these maturing males when fish are either spawned in captivity or released from hatcheries. The underlying factors regulating the age of maturity in salmonids involve both genetic and environmental factors (Rowe and Thorpe 1990a,b). Because genetic selection should be minimized in a captive broodstock program for depleted stocks, rearing strategies that minimize expression of the trait are needed. Our approach has been to systematically identify factors that influence age of maturation and determine seasonal periods when maturation is initiated in spring chinook salmon. This information is being used to develop diets and growth regimes that reduce the incidence of early male maturity, provide sufficient stored energy for appropriate life-cycle transitions, sustain development of gametes in adult fish, and achieve target size for release as adult fish.

Four major experiments that examined the relationships among growth rate /size, fatness and the incidence of male sexual maturation at 1+ years of age in spring chinook salmon have been conducted. Spring chinook salmon from the Yakima River stock were used in the first experiment, while the Willamette River stock was used for the others. Fish were sampled approximately monthly to determine the level of body fat and state of sexual maturation in all experiments. In the first study, body weight was controlled and fat levels were manipulated by varying the protein:energy content of the diet. A significant positive correlation of the percent of males maturing at 2 years of age with body fat levels was found (Shearer and Swanson 2000). In addition, increases in reproductive hormones that were associated with the onset of spermatogenesis occurred in the fall, nearly 10 months prior to when the males fully matured. In the second experiment in which both size and body fat levels were manipulated, growth rate or size one year prior to maturation was the primary factor affecting maturation at 2 years of age (Silverstein et al. 1998). Fat levels significantly affected maturation rates, but only in groups with smaller

fish. Subsequently, the third and fourth studies were conducted to determine the threshold of size or growth rate that influences the onset of male maturation. In the third study (Shearer et al. 2000), fish were fed high protein, low fat diets of graded ration levels. Fish size during the first autumn ranged from 50 - 100 g. At age two, 65 - 90% of the males matured, with the rate of maturation increasing with size. Therefore, even though size or growth rate clearly influenced the rate of male maturation, the threshold had clearly been exceeded in most fish. In the fourth study, fish were reared on graded rations of a commercially available diet and targeted a much lower body size for the first year of rearing than the first study. The relationship between size after the first year of rearing and the rate of early male maturation for all four studies are shown in Figure 1. For spring chinook salmon, the combined data suggest that a growth trajectory that produces a fish of 10 g body weight in the first year of rearing should be used to minimize maturation in the subsequent year. This target size is similar to that of juvenile wild chinook salmon in the Yakima River Basin (D. Larsen, NMFS, personal communication).

From these studies, it was concluded that maturation is initiated during late fall or early winter, nearly 10 months prior to spawning in September. Growth rate and/or size, appears to be the most important factor determining whether a male chinook salmon will initiate maturation since the proportion of males maturing in September at age 2 is positively correlated with size the previous December (Figure 1). Body fat levels influenced the proportion of males maturing, but only in smaller fish (Figure 2, and Silverstein et al. 1998). Our results indicate that it may be possible to alter current rearing practices to reduce the incidence of maturation in 1+ age male spring chinook salmon, by reducing growth during critical periods of the life cycle such as the first fall in fresh water. However, to achieve reduced growth during the first year it may be necessary to delay emergence of fry using reduced water temperature during egg incubation.

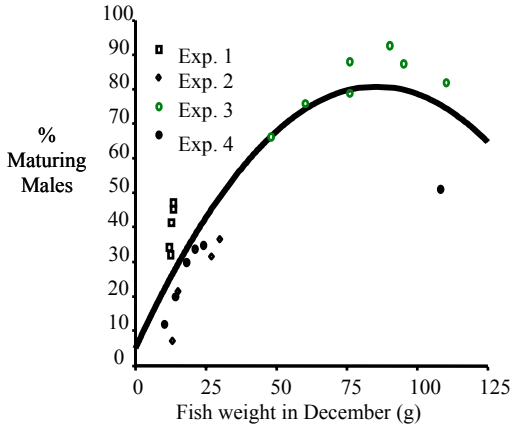


Figure 1. The relationship between fish weight in December (0+ age) and the incidence of male maturation at age 1+. Data are from four experiments conducted between 1993-2001. Exp. 1 (Shearer and Swanson 2000), Exp. 2 (Silverstein et al. 1998), Exp. 3 (Shearer et al. 2000), Exp. 4 (Shearer et al. unpublished).

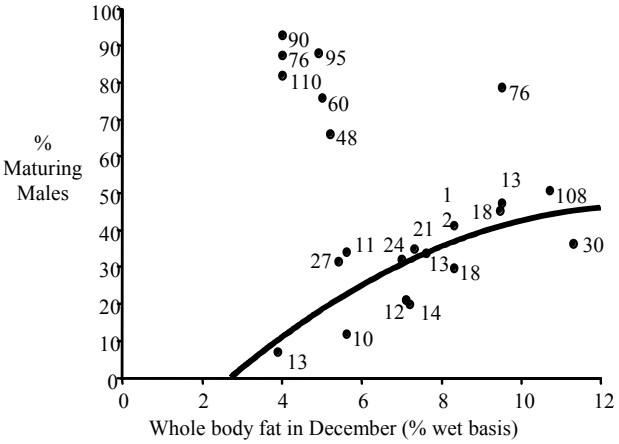


Figure 2. The relationship between whole body fat in December (0+ age) and the incidence of male maturation at age 1+. Body weight of fish are shown adjacent to data points. Data are from four experiments conducted between 1993-2001 as in Figure 1. Note that in larger fish, the incidence of maturation is not influenced by body fat level.

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**Effects of photoperiod, temperature and GnRHa treatment on the timing of spawning and egg and sperm quality in atlantic salmon (*Salmo Salar* L.)**

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*EXTENDED ABSTRACT – DO NOT CITE*

In the farming of Atlantic salmon, advancing the natural spawning season is advantageous in the current production of 0+ smolts, while off-season egg production would greatly facilitate year-round smolt production. It may also be desirable to synchronize or advance the spawning of returning fish in wild stock enhancement hatcheries. Photoperiod appears to be the most important environmental cue controlling the timing of spawning in salmonids, and photoperiod manipulation can be used to alter the timing of spawning within the normal spawning season (e.g. Bromage et al. 2001). However, water temperature can also affect the timing of spawning in salmonids, with high water temperatures during the spawning season delaying or inhibiting spawning (Taranger and Hansen 1993, Pankhurst and Thomas 1998). This apparent inhibition has been overcome by injections of Luteinizing Hormone Releasing Hormone Analogues (LHRHa) in rainbow trout maintained at 18ºC (Pankhurst and Thomas 1998), but not in Atlantic salmon maintained at 16ºC (King and Pankhurst 2000).

In a study with previously immature Atlantic salmon, exposure to an accelerated photoperiod regime (AP; continuous light [24:0 LD] from February, thereafter short day [8:16 LD] from May) advanced the median ovulation date by approximately 5 weeks when compared to controls reared under simulated natural photoperiod (NP) (Taranger et al. 2000). However, ovulation was desynchronized in the AP group under ambient temperatures, and egg survival to eyed stage was significantly lower than in the NP group also reared under ambient temperature. n contrast, exposure to cooled water (temperature decreased from 14º to 8°C on September 6, and maintained at approximately 6°C below ambient) synchronized and advanced ovulation in both the AP and NP groups when compared with fish maintained at ambient temperature (Figure 1). Exposure to the cooled water also resulted in a higher egg survival percentage in the AP group, equal to that of the NP group. This study indicated that exposure to cold water prior to spawning advances and synchronizes ovulation in Atlantic salmon, and that the combined use of photoperiod and temperature can be used to control the spawning time in salmon broodstock without affecting egg quality.

In another study, previously immature Atlantic salmon broodstock were exposed to three different temperature regimes on September 28, 2001 (high; 14º-16ºC, ambient; decreasing from 11º to 5ºC between September and December, and cold; 3º-7ºC). On October 5, 2001 each of the temperature treatments were divided further into three subgroups; one group receiving a GnRH analogue treatment (biodegradable microspheres containing [D-Ala6, Pro9NEt]-GnRH were injected in the dorsal muscle at a dosage of 50 μg $kg^{-1}$ body weight, Mylonas et al. 1995), a sham treatment group (microspheres only), and an untreated control. All groups were reared under simulated natural photoperiod for the duration of the experiment. Fish from the GnRHa treatment and sham control groups were slaughtered after four weeks, on October 31, the effective duration of the GnRHa treatment. Untreated control fish were slaughtered on December 12. Both ovulation and spermiation were completely inhibited in the high temperature group, while the cold water treatment slightly advanced spawning in both sexes. Treatment with GnRHa both advanced and synchronized spawning in male and female fish at all temperatures, overriding the inhibition induced by elevated temperatures. The synchronizing effect of GnRHa was strongly pronounced in both sexes (Table 1). In the cold and ambient groups all GnRHa males had running milt within the first week, three weeks before the control and sham groups. All warm-water GnRHa males were producing sperm during the second week. Additionally, GnRHa effected a four to fivefold increase in the total volume of milt in males when compared with controls. In females, 75% (warm) to 90% (ambient) of GnRHa fish spawned within the four-week GnRH trial, while only 20% to 30% of the control and sham fish spawned during this period. Survival of eggs to eyed stage was slightly higher in the cold water group than in ambient, while eggs of GnRHa treated fish generally exhibited lower survival rates than sham and untreated controls (Table 1). However, egg survival in the warm water GnRH group was over 60%, which is acceptable for most farming situations. These results appear to strengthen the previous findings on the positive effects of a cold water shock on broodstock and the resulting egg quality. Additionally, the results of the GnRHa trial reinforce its potential as a tool in salmon broodstock management.

**Got Milt? Lack of Adequate Milt Production in a Captive Broodstock Program for Redfish Lake Sockeye**

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*EXTENDED ABSTRACT – DO NOT CITE*

The National Marine Fisheries Service (NMFS) and the Idaho Department of Fish and Game (IDFG) cooperatively established a captive broodstock program for Redfish Lake sockeye salmon in 1991 in an effort to ward off extinction of this endangered population. All returning wild adults from1991-1998 (n =16), and a portion of returning program adults in 1999-2001 (n = 290) were trapped and spawned by IDFG personnel. The eggs were divided between NMFS and IDFG facilities for full term captive rearing (3 – 4 years). The maturing adults were spawned, crosses made, and the resulting eggs returned to Idaho for release as eggs, fry, smolts, and adults. Captive broodstock spawning began in 1994 and has occurred every year since with the exception of 1995. Each female's eggs are divided into two separate egg lots, with each lot fertilized by a different male. Each year survival to the eyed egg stage has averaged only about 60% with a range from 0 – 98%.

Several reproductive problems have been noted in the captive program. First, the onset of maturation in the captive population is delayed 2 – 4 weeks relative to the wild founding population (Bjornn et al. 1969). Second, male and female maturation is asynchronous, with males maturing 2 – 3 weeks later than females. Third, there has been a marked decrease in the volume of milt produced by captive males in the NMFS component of the Redfish Lake sockeye program. Males in the captive program spawn as 2-, 3-, and 4-year-old adults. Milt volume increases with maturation age and ranges from 0.5 - 8.0 mls per male. In the 2001 spawning a negative male effect on egg viability was evident in at least 50% of the crosses.

Inadequate milt production limits the range of genetically desirable crosses, and is a suspected contributor to high variability and low (< 60%) average egg viabilities. Males are experiencing delayed and incomplete spermiation. Although the reasons for this are not clear, they may be due to a lack of appropriate environmental cues, such as photoperiod, exercise, and temperature in the final months of maturation. Photoperiod is probably not a factor as the NMFS program mimics a natural photoperiod regimen, utilizing a combination of ambient and artificial lighting. There is virtually no exercise current present beyond low tank velocities (about 1 body length/second), and water temperature is a constant 10°C with no diurnal fluctuations or gradual decrease in

temperature prior to maturation. Although GnRHa implants have been used to increase milt production they have met with mixed, and unpredictable results.

Future approaches to solving this problem may include: 1) providing an exercise regimen for several months prior to maturation to emulate upstream migration (anadromous adults swim 900 miles upstream from the Pacific Ocean to Redfish Lake), 2) diurnal temperature fluctuation or temperature drop prior to maturation, and 3) adjusting the GnRHa implant strategy by either increasing the dosage, or implanting earlier.

Despite the above concerns, the NMFS captive brood program has provided 870,000 eggs and 249 adults for recovery of this ESA listed stock. The IDFG and NMFS captive broodstock programs have returned 290 anadromous adults to Redfish Lake during the past 3 years.

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**Factors Affecting Captive Salmonid Embryo Survival**

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*EXTENDED ABSTRACT – DO NOT CITE*

Two components important for the survival of salmonid embryos are the quality of the gametes produced by the parents and the environment during early rearing. It is particularly critical with captive broodstocks from declining or endangered populations to appreciate how factors derived from these two components can affect embryo survival. This presentation will review some of the known biological factors that affect embryo survival and identify areas that could be crucial but remain unstudied. These factors can be organized into two categories that occur sequentially, those that affect fertilization, and those that impact early development. Fertilization will be defined as embryological events leading to the formation of a zygote (single cell) and ending after the first cleavage division. Early development will encompass events that occur after the first cleavage division and extend up to emergence from the egg (i.e., hatching).

Factors affecting fertilization relate directly to the newly formed gametes and the process of zygote formation. Obviously, a strong genetic influence will be present with some fish producing higher quality gametes than others. It is understood that the holding conditions (e.g. temperature, photoperiod, diets, etc.) of the adults and their health need to be considered too, but these aspects will not be covered here. A greater difference in gamete quality may occur in captive broodstocks compared to domesticated stocks of fish. Data from hatchery-raised rainbow trout still show considerable variability in embryo survival, and it is not clear what this is due to, but a significant genetic component is expected. This could affect either the eggs or sperm, although if poor sperm quality is known this can be overcome by increasing sperm concentrations during in vitro fertilization procedures. The condition of the eggs prior to fertilization is also critical and more difficult to overcome if deemed to be sub-fertile. Studies have shown that eggs remaining in the body cavity of the female for extended periods of time produce fewer fertilized eggs; the condition of over-ripening. The ovarian fluid can contain high bacterial loads and the phenomenon of over-ripening may be due to bacterial contamination, although this remains to be investigated. More frequent assessment of ovulation can be used to mitigate over-ripening.

During the period of early development we have shown that the majority of the variability in embryo survival in rainbow trout is due to the female. Experiments using a single-pair mating design revealed that the egg has a much more significant impact on embryo survival after fertilization than that derived from the sperm. This implicates some property of the fertilized egg itself is strongly influencing early development. The

general chemical composition of the eggs of some salmonid species have been investigated and no distinct pattern of differences in amino acids, minerals, lipids, or vitamins have been related directly to embryo viability. However, the genetic material (DNA and RNA) within the egg that programs development has received less attention and is an area that we have begun to study. In rainbow trout, it was shown that maternally derived messenger RNA (mRNA) is important for the first few days of early development before the embryonic genome takes over. We speculate that alterations in developmental programming through maternal mRNA and subsequent effects on embryonic DNA could cause problems with embryo survival. The challenge will be to determine the root cause(s) of altered or inappropriate genetic programming.

**Spreading the Risk: Managing and Evaluating Reintroduction Strategies for Redfish Lake Sockeye Salmon.**

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*EXTENDED ABSTRACT – DO NOT CITE*

Precipitous declines of Snake River sockeye salmon led to their Federal listing as endangered in 1991. In that same year, the Idaho Department of Fish and Game (IDFG) initiated a captive broodstock program to maintain Snake River sockeye salmon and prevent species extinction. The ultimate program goal is to reestablish sockeye salmon runs and to provide for sport and treaty harvest opportunities. In the near-term, the program is focused on preventing further population loss, maintaining population genetic integrity, and rebuilding population numbers.

Since the inception of the program in 1991, all returning anadromous adult sockeye salmon (16 wild fish), several hundred Redfish Lake wild out-migrants, and several residual sockeye salmon adults have been captured and used to develop captive broodstocks at the IDFG Eagle Fish Hatchery and at National Marine Fisheries Service (NMFS) facilities in Washington State. Adaptively managed, the program generates hatchery-produced eggs, juveniles, and adults for reintroduction to Stanley Basin, Idaho waters. In addition, emphasis is placed on the annual development of genetically diverse broodstocks. Program captive broodstock techniques reflect the region's best protocols for maintaining maximum genetic diversity, survival, and production success. Fish culture variables (e.g., broodstock mating designs, fish survival, maturation success, fecundity, egg survival to eye, and fish health) are continuously monitored and evaluated to insure maximum program success. Juvenile out-migrant monitoring (using PIT tag technology), adult return monitoring, and adult sonic telemetry studies provide information critical for the evaluation of program reintroduction strategies. Program methods and results undergo constant review through the Stanley Basin Sockeye Technical Oversight Committee (SBSTOC), a team of technical experts assembled to review program results and to guide program direction.

Through 2001, the IDFG and NMFS hatchery programs have produced in excess of 720,000 pre-smolts, 120,000 smolts, 690 adults, and 295,000 eyed-eggs for reintroduction to Stanley Basin lakes and tributary streams. From this production, approximately 260,000 hatchery-produced, juvenile sockeye salmon have emigrated from Stanley Basin waters. In 1999, the first hatchery-produced anadromous adults returned to the Stanley Basin. In that year, seven age 3 adults (six males and one female) returned to spawn. In 2000, the program experienced its first significant return of hatchery-produced adults. Two hundred fifty-seven sockeye salmon returned to collection facilities on

Redfish Lake Creek and the upper Salmon River at the IDFG Sawtooth Fish Hatchery. The majority of year 2000 adult returns were released to the system for natural spawning. In 2001, 26 hatchery-produced adults returned to collection facilities in the Stanley Basin.

The development of egg and fish reintroduction plans has followed a "spread-the-risk" philosophy incorporating several release strategies and multiple lakes. The first releases age 0, hatchery-produced juvenile sockeye to Stanley Basin lakes occurred in 1994. Since that time, Redfish Lake has received pre-smolt plants in each year the program has operated. Two pre-smolt release strategies have been employed in Redfish Lake: a fall direct-lake release, and a fall release from a net pen environment. In 1995 and 1997, Pettit and Alturas lakes were incorporated in annual release and evaluation activities. Both lakes have received mid-summer and fall, direct-lake introductions of pre-smolts. Pre-smolt release groups are generated from eggs produced at the IDFG Eagle Fish Hatchery and the NMFS-operated Burley Creek Hatchery. Rearing through release takes place at the IDFG Eagle and Sawtooth fish hatcheries. All fish are adipose fin-clipped and a portion PIT-tagged to facilitate over-winter survival and out-migration evaluations.

Of the two pre-smolt release options used to date in Redfish Lake (fall direct-lake and fall release from net pens), fish generated from fall direct-lake releases over-wintered and out-migrated significantly better than net pen fish in three of the five years of paired investigations ( $^2$ tests for independence, $p < 0.0001$). In addition, Alturas and Pettit lake outmigrants produced from fall direct-lake release groups have performed consistently well. Paired summer direct-lake and fall direct-lake pre-smolt releases are currently being evaluated in Alturas and Pettit lakes. First year results indicate that fish produced from the fall direct-lake release strategy performed significantly better than fish produced from the summer direct-lake release strategy ($p < 0.0001$).

Out-migration evaluation results are now shaping the development of annual release plans for the captive broodstock program. Summer direct-lake releases in Alturas and Pettit lakes and net pen releases in Redfish Lake have not been entirely abandoned, but emphasis is being placed on the more successful fall pre-smolt release option.

**Redd to spawner evaluation of adult Atlantic salmon stocking**

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*EXTENDED ABSTRACT – DO NOT CITE*

Wild Atlantic salmon (*Salmo salar*) runs in US waters have experienced significant declines in abundance including the extirpation of numerous stocks. Today, only eight remnant populations remain in the USA; all are located within the State of Maine (Colligan *et al.* 1999). These populations have also experienced dramatic declines in abundance and it is estimated that between 86 and 114 total mixed origin fish (wild and hatchery reared) returned to these eight rivers to spawn in 2001. In 2000, these populations were formally listed as endangered under the Endangered Species Act.

Historically, the stocking of various stages of Atlantic salmon has been used to artificially augment natural production within these rivers. In 1992, the stocking program was modified to a river-specific approach (stocking progeny originating from broodstock captured as parr from each specific river), with a focus on fry stocking. Since its inception, fry have accounted for between 94-100% of the stocked fish. In 1997, as an alternative to fry stocking, a two-year feasibility study was initiated between private aquaculture companies and Federal and State of Maine management agencies. Atlantic salmon were reared to maturity in salt water and stocked in Maine Rivers in an effort to enhance natural spawning escapement. In 1997, river-specific broodstock from three Atlantic salmon populations were held and spawned in a Federal hatchery. Eyed-eggs were transferred to private aquaculture companies and were reared to the smolt stage in freshwater; smolts were then transferred to marine sea-cages and reared to maturity. This process was repeated in 1998 with river-specific broodstock from four rivers. A comprehensive stocking and assessment plan was also developed to gauge the effectiveness of this adult stocking effort in supplementing these depressed populations.

During October 2000, 1,038 marine-reared, mature adult Atlantic salmon were released into the Dennys, Machias, and St Croix Rivers. Prior to stocking, all adults were

weighed, measured, PIT tagged and a genetic sample was taken. During the stocking process the adults were physically handled a minimum of 6 times, which anecdotally resulted in increased stress and physical damage to the fish. In addition, 70 Dennys River origin adults were ultrasonically tagged and 15 ultrasonic receivers were deployed throughout the Dennys River to evaluate pre-spawning and over winter migratory behaviors. Telemetry data indicated that these stocked adults freely moved throughout the drainage and actively sought appropriate spawning habitat. Post stocking assessments documented a significant increase in redd production attributable to these stocked adults, but negative results from follow-up fry emergence investigations have since called into question the reproductive success of these fish as well as the viability of gametes.

In 2001, 703 marine-reared, mature adults were released into these same three drainages. These adults were sampled in a similar manner as the year 2000 fish, however stocking logistics were modified to help reduce the number of times each adult was handled. These actions presumably resulted in a lower stress level for the pre-spawning adults as well as less external physical damage. Telemetry investigations were duplicated and we expect these data to provide insights into pre-spawning migratory behavior, spatial and temporal behavioral differences and over-winter residency of the stocked adults. Additionally, laboratory and hatchery-based assessments on the viability of gametes produced by these stocked adults were carried out. Preliminary analyses indicate that the 2001 stocked adults are responsible for a significant increase in the number of redds documented within each recipient river and that their gametes are viable. Preliminary fry trapping results aimed at assessing the fry emergence rate from these redds on two rivers have been contradictory. Population surveys of parr (electrofishing) and out-migrating smolts (trapping) will also be conducted and an adult capture facility will be operated annually. Genetic samples will be obtained during all future population assessment activities and will allow for the partitioning of samples by origin via parentage analysis (wild spawning, fry stocked, or adult stocked).

In summary, a total of 1,741 marine-reared, river-specific, mature Atlantic salmon adults were stocked into three Maine rivers over a two-year period (Table 1). Evaluations of this unique supplementation method are ongoing and will provide valuable information regarding the effectiveness of this technique in supplementing natural reproduction within these depressed populations. Initial results indicate that the stocking of marine-reared mature adults may be a management tool capable of artificially increasing the number of adult spawners and egg deposition until a time when environmental conditions improve and natural spawning escapement increases.

Table 1. Numbers of adult, marine-reared, mature Atlantic salmon stocked into three Maine Rivers in 2000 and 2001. The individuals were spawned at a Federal hatchery and reared for 2 sea-winters at a commercial netpen facility as part of a two-year joint venture between Federal and State of Maine natural resource agencies and the Maine Commercial Aquaculture Industry.

| | 2000 | | | | 2001 | | | | *Grand* |
|---|---|---|---|---|---|---|---|---|---|
| Drainage | Males | Females | Unknown | Total | Males | Females | Unknown | Total | *Total* |
| Dennys | 57 | 55 | 0 | 112 | 25 | 50 | 0 | 75 | *187* |
| Machias | 93 | 83 | 0 | 176 | 39 | 65 | 0 | 104 | *280* |
| St Croix | 338 | 412 | 0 | 750 | 212 | 305 | 7 | 524 | *1,274* |
| Total | 488 | 550 | 0 | 1,038 | 276 | 420 | 7 | 703 | *1,741* |

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**Testing the Ability of Captive-Reared Chinook Salmon to Spawn Naturally**

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EXTENDED ABSTRACT ONLY – DO NOT CITE

In a captive rearing program, the maturing adults are returned to spawn in their natal stream. The Idaho Department of Fish and Game began its captive-rearing program for chinook salmon to determine the potential usefulness of this strategy in sustaining depressed populations until the underlying causes for their decline are addressed. Three Salmon River tributary streams are included in the project including the West Fork Yankee Fork Salmon River, the East Fork Salmon River, and the Lemhi River.

This program is part of a larger effort evaluating two different captive culture techniques: captive rearing and captive brood stocking. The two methods are initially similar, but diverge when the fish mature. Both techniques rear naturally spawned fish in a hatchery until sexual maturity. In a captive brood stock program the adults are spawned in the hatchery, and the resulting progeny are released in their parent's natal stream. In a

We believe the captive-rearing approach has several advantages over brood stocking. Captive rearing allows natural selection to occur while study animals compete for mates. The time fish spend in a hatchery environment is minimized, presumably along with the domestication selection present there (Reisenbichler and Rubin 1999). This technique also allows managers to better manage the effects of a captive breeding program on the effective population size of the target population (Ryman and Laikre 1991). A final advantage of captive rearing is that less facility space is required since smolts are not retained on station.

The chinook salmon captive rearing program has four primary objectives that include developing techniques to rear chinook salmon to maturity in captivity, producing adults with characteristics that facilitate reproduction with wild fish, monitoring their reproductive success, and maintaining study populations and their genetic identities.

Several challenges remain before captive rearing becomes a viable management tool. Captive-reared fish have consistently spawned several weeks later than wild fish in the same stream. Captive-reared fish have been significantly smaller than wild fish. Disease outbreaks including bacterial kidney disease, whirling disease, and parasitic copepods infestations have been responsible for a large percentage of project mortality, and finally, precocial male maturation is becoming increasingly more common.

In response to these limitations, we have altered our culture techniques or are conducting experiments to find ways to correct them. We are addressing delayed spawn timing by using ultrasound to identify maturing fish earlier in order to transfer them back to

freshwater nearer the time their wild counterparts are entering the Columbia River. We are also conducting experiments to determine if pre-release holding on chilled water will advance spawn timing. Many of the disease, and possibly growth, issues have been corrected by collecting eyed-eggs instead of juveniles. Eggs are not susceptible to the causative agent of whirling disease (Markiw 1991) or the parasitic copepods present in the Lemhi River. Mortality from bacterial kidney disease has also declined since we changed collection methods. Improved health and no forced switch from a natural to a hatchery diet has led to improved growth rates and greater survival in groups collected as eyed-eggs. A potential downside to this change is that, along with improved health and growth, precocial male maturation has increased. Attempts to reduce this include changes in diet formulation, daily ration, and the use of chilled water.

Despite these limitations, captive-reared chinook salmon released into the West Fork Yankee Fork Salmon River in 2001 (N=89) displayed behavior generally similar to that of wild fish while spawning. Variables monitored included spawn timing, the frequency of male courtship, female digging frequency, and male aggression. Although captive-reared fish spawned later than wild fish, those from the chilled water group tended to spawn earlier than those from the ambient temperature group. Captive-reared females initiated eight redds between 8/30/01 and 9/5/01, and of these five were from the chilled water group. Some redds in this group were spawned early enough that wild males were still present, which allowed comparisons between captive-reared and wild males to be made. Seven captive-reared females initiated redds between 9/6/02 and 9/17/02, with only one being from the chilled group. Behavioral observations indicated captive-reared males displayed the same courtship patterns as spawning neared, but often at reduced levels (Figure 1). Captive-reared males were, however, much less aggressive than wild males, and were always dominated by them where both were present. Female dig frequency and timing followed the same pattern observed in spawning coho salmon *Oncorhynchus kisutch* (Berejikian, et al. 2001), and was the same regardless of male rearing history (Figure 2).

Survival to the eyed stage and fertilization of eggs spawned by captive-reared females were estimated by sampling several redds. Eggs were collected from five of eight redds sampled, and survival averaged 68.3% (range 29.0%–88.9%). Results from one redd were discarded because it was constructed on a decayed log concealed by a layer of gravel.

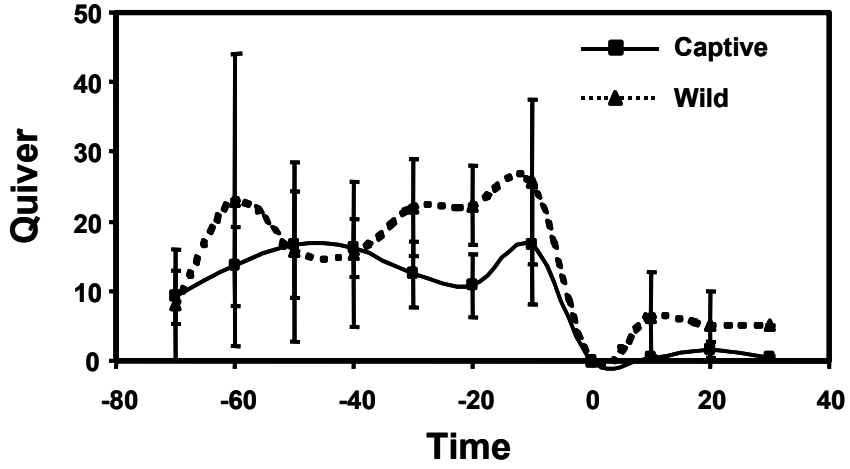


Figure 1. Mean (± S.E.) frequency of a male courtship behavior during 10 min intervals by captive-reared and wild chinook salmon observed spawning with captive-reared females in the West Fork Yankee Fork Salmon River in 2001. Time zero is spawning, and negative and positive numbers are minutes prior to and post spawning, respectively.

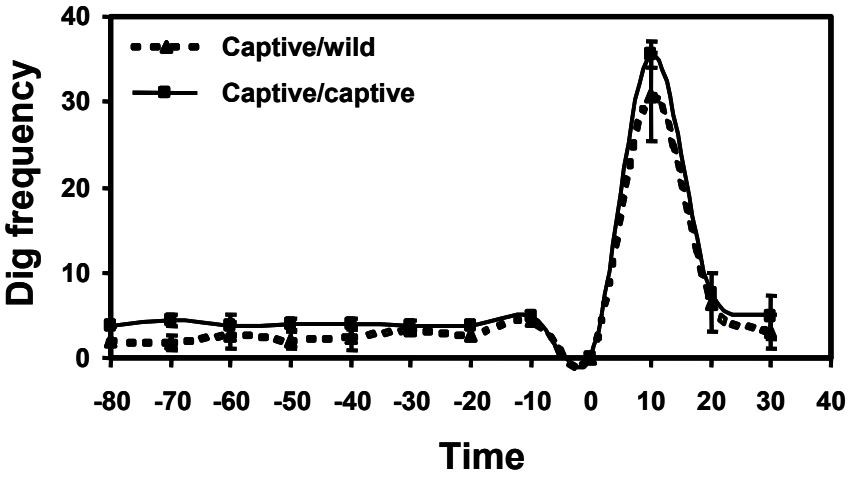


Figure 2. Mean (± S.E.) digging frequency by captive-reared, female chinook salmon observed spawning with captive-reared and wild males in the West Fork Yankee Fork Salmon River in 2001. Time zero represents spawning, and negative and positive numbers are minutes prior to and post spawning, respectively.

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**Rearing and Reintroduction Strategies for Salmonid Captive Broodstocks: Implications for Olfactory Imprinting, Homing, and Straying**

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*EXTENDED ABSTRACT – DO NOT CITE*

The ultimate goal of most salmonid captive broodstock programs is the re-establishment of self-sustaining wild populations in their ancestral rivers. Strategies for rearing and reintroducing captively-reared fish that minimize the phenotypic and genetic effects of culture and maximize survival are being developed and tested for a number of salmonid species. Different rearing and release strategies may also impact the ability of juvenile salmon to imprint and therefore successfully home back to waters targeted for re-establishment of wild populations. Pacific salmon imprint to odors associated with their natal stream as juveniles and then later use these retained odor memories to guide the final phases of their homestream migration. Salmon that do not experience their natal water during appropriate juvenile stages are more likely to stray to non-natal sites. By identifying developmental periods and environmental conditions that are important for olfactory imprinting, rearing and release strategies for each salmon species can be developed to lower stray rates in both production and recovery hatcheries.

Hatchery rearing does not necessarily result in increased levels of straying but certain hatchery practices do clearly increase stray rates (Quinn 1993). In particular, fish that are reared and released from a single site generally stray less than fish transported and released off-site. The effect of transport on homing is particularly important in Captive Broodstock programs because they frequently do not have appropriate rearing facilities available at the ancestral site or must rear fish at several sites to avoid the risk of cataclysmic events at a single facility. In general, when fish are transported and released away from their rearing site, 1) stray rates are inversely related to distance transported from the rearing site; 2) salmon released out of the watershed in which they are initially reared generally stray less than salmon released within system; 3) the importance of acclimation prior to release to improve imprinting/homing has not been demonstrated (Lister et al 1981; Vander Haegen and Doty 1995; Quinn 1993; Dittman unpub.).

One example that illustrates some of the challenges for a captive broodstock/conservation hatchery program is the Redfish Lake sockeye salmon captive broodstock program. Snake River sockeye salmon were listed as endangered by NMFS in 1991 and in that same year the IDFG initiated a captive broodstock program with the ultimate goal of re-establishing sustainable sockeye runs to Stanley Basin waters. Captively-reared population numbers have increased to the point that since 1993 fish have been re-introduced annually into the Stanley Basin. To avoid unanticipated negative consequences of any one reintroduction approach, the IDFG, in conjunction with the

Stanley Basin Sockeye Technical Oversight Committee (SBSTOC), has adopted a "spread the risk " strategy for reintroducing sockeye back into the wild that includes planting of eyed eggs, net pen and direct lake releases of pre-smolts, smolt releases and releasing captively-reared adults to spawn naturally (Figure 1). Fish for these releases were reared at several out-of-basin facilities (NMFS hatcheries at Manchester and Big Beef Creek, Washington; IDFG hatcheries at Eagle and Sawtooth, Idaho, ODFW Bonneville hatchery) because there were no appropriate Stanley Basin facilities and to avoid the risk of cataclysmic events at a single facility (Figure 1). In some instances fish were transferred several times at different life stages between facilities and some groups didn't experience Stanley Basin waters until they were released as smolts.

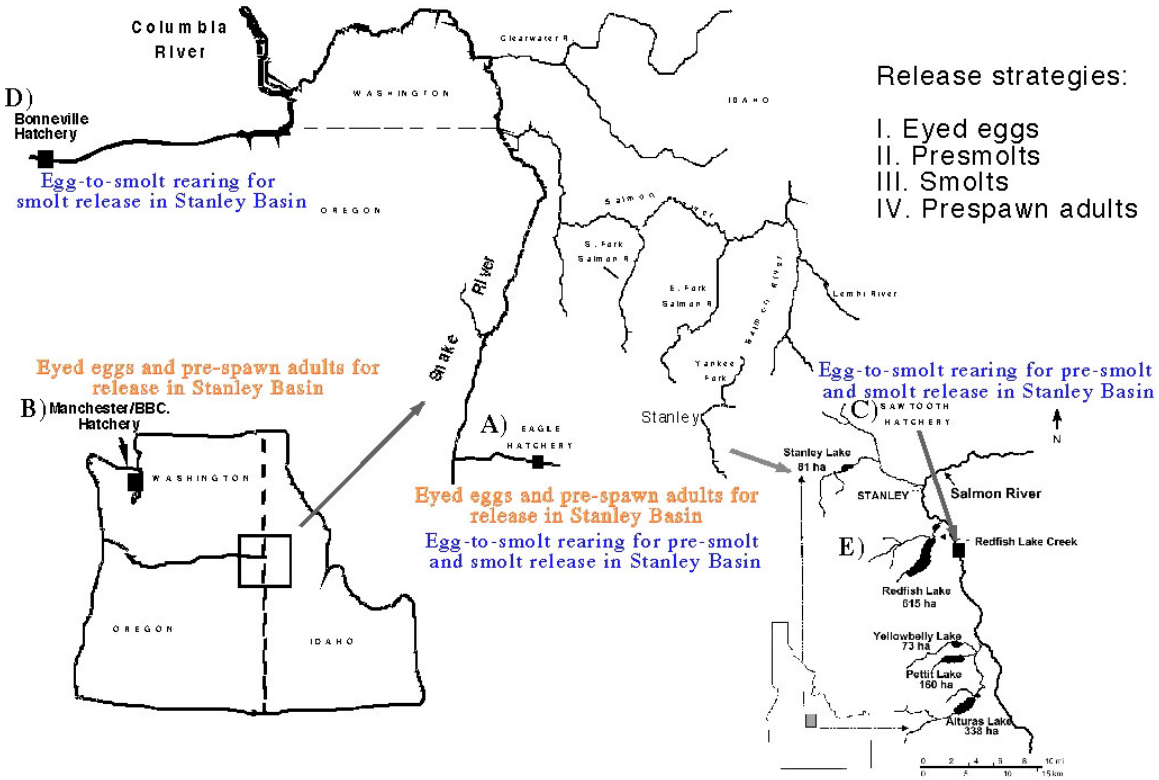


Figure 1. Rearing hatcheries and release strategies for Stanley Basin Sockeye Captive Broodstock Program. Embryos for eyed-egg plants and pre-spawn adults for natural spawning releases into Stanley Basin (E) were reared at the (A) IDFG Eagle hatchery and (B) NMFS BBC/Manchester hatcheries. Pre-smolt and smolt release fish were also reared at the (C) IDFG Sawtooth hatchery and (D) ODFW Bonneville hatchery. Different strategies often involved multiple movements of fish between hatcheries prior to release.

Empirical studies have provided some general rules regarding the effect of hatchery rearing and release strategies on straying, but in many cases, differences between species, watersheds, physical environment of the hatchery, release timing and location, and even basic assumptions about what should be regarded as successful homing may mask the underlying processes that are critical for imprinting and homing. Ultimately, determining the critical development periods and environmental conditions for imprinting for the different salmon species will be crucial for the development and implementation of rearing and release strategies that will maximize survival without increasing straying. To determine the critical period(s) for imprinting for sockeye salmon, juvenile salmon are being exposed to known odorants (PEA, amino acids) at key developmental stages that

parallel the redfish lake reintroduction strategies and will be subsequently tested for development of long-term memories of these odorants. Assessment of imprinting will entail a combination of behavioral, physiological and molecular measures of olfactory learning. Physiological assessment of imprinting will involve measuring the olfactory sensitivity to exposure odorants using electro-olfactograms (EOG), a simple electrophysiological technique that measures the summated responses of many receptor neurons in the olfactory epithelium. Because the olfactory epithelium is apparently sensitized to specific odors during imprinting (Nevitt et al. 1994, Dittman et al. 1997), the EOG may provide a rapid, sensitive, and cost-effective method for assessing sensitization to imprinted odorants.

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**A Review of Steelhead and Rainbow Trout Research at Little Port Walter, Alaska**

Frank Thrower

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*EXTENDED ABSTRACT - DO NOT CITE*

The paper reviews research on steelhead and rainbow trout initiated at the Little Port Walter Research Station in 1996 by the Auke Bay Laboratory as a result of funding from the Protected Resources Division of the National Marine Fisheries Service. The primary impetus for the funding was to conduct long term genetics research related to inbreeding and outbreeding depression in steelhead.

As part of this research 75 families of steelhead (first generation from wild) and resident rainbow of the same stock maintained in freshwater for 70 years in a wild state are being cultured. Comparisons are being made between pure stocks and hybrids in captivity, and in a sea ranched component. The presentation will review growth, survival and maturation differences between groups.

## PART II. DISCUSSION SUMMARY

### Day 1: June 25, 2002

***1. Greetings and Introductions.***

Workshop chair Barry Berejikian of NMFS welcomed everyone to the workshop, and led a review of today's agenda. He then introduced the first speaker of the day.

The following is a summary, not a verbatim transcript, of the discussion that followed each of the workshop presentations. Any questions about this summary should be directed to Berejikian at 360/871-8301.

***2. Keynote Presentation: History of Captive Broodstock Programs and Future Challenges.***

Tom Flagg of NMFS led this presentation. In response to a question, Flagg said that, obviously, he would prefer to see higher survivals in captive broodstock programs; while egg viability continues to be a problem, survival has been high enough – 50%-65% – to provide the number of animals needed for outplanting and captive rearing.

In response to another question regarding the need for captive broodstock programs to combine natural and hatchery reproduction, Flagg said that one of the goals of these programs is to learn from previous mistakes. We don't want any animals we release to the wild to carry any environmental or genetic baggage, he said, but, rather, to seamlessly meld into the wild returning spawning population and to contribute positively to the population. Is vigor a synonym for fitness? one participant asked. It is a synonym for reproductive viability, Flagg replied.

***3. Session 1: Genetics Issues in Captive Broodstocks.***

This session was moderated by Jeff Hard of NMFS, who provided a brief introduction to the topic and to each of the presenters.

***A. Balancing Genetic Risks and Demographic Gains in Captive Broodstock Programs***. Craig Busack of WDFW was the presenter for this topic. Afterwards, one workshop participant asked about fine-scale genetic structure in the context of broodstock programs; Busack said he considers mate selection to be one of the most important factors in the success of broodstock programs. There are two ways to look at genetic diversity in the hatchery: quantity -- the sheer number of spawners -- vs. quality – the actual genetic processes and selection taking place, and how it can be disrupted during broodstock programs, he said. The more we intervene, the greater the potential for that problem, Busack agreed.

***B. Genetic Considerations in Broodstock Management***. Kerry Naish of the University of Washington was the presenter for this topic. Following her presentation, one participant asked whether it is possible that one of the assumptions we start out with -- one-on-one mating – is flawed? Wouldn't it be better, from the standpoint of genetic diversity, to utilize two-on-two or three-on-three matings? he asked. I won't argue with that, Naish replied. In response to another question, Naish said one thing that isn't known is how reversable domestication selection may be. One idea is that we put out as much population diversity as possible, then let wild selection do the rest, she said.

***C. Examples of Genetic Management in Captive Populations: Redfish Lake Sockeye and Salmon River Chinook Salmon***. Matt Powell led this presentation. Afterward, Hard asked that questions for Powell be held until the final "Synthesis and Discussion"segment of this session.

***D. Inbreeding and its Consequences: What Do We Know and What Do We Need to Know***? The presenter for this topic was moderator Jeff Hard. Following his presentation, questions were again held until the "Synthesis and Discussion" segment of this session.

***E. Do We Need to Worry About Domestication Selection?*** Mike Ford led this discussion. Again, questions were held until the "Synthesis and Discussion" portion of this session.

***F. Synthesis and Discussion***. Evidence of sibling recognition in wild fish exists, noted one participant. Has anyone looked at all at inbreeding in wild fish? Certainly not in salmonids, that I'm aware of, Hard replied. The potential for inbreeding is certainly very high in some of these wild populations; we don't know whether anadromous Pacific salmon may or may not be more resistant to inbreeding, Hard said. We hope to design such a study at the Science Center, he added.

Can you discuss the value of supplementation programs as opposed to the value of other restoration efforts, such as habitat restoration? another participant asked. In my opinion, captive broodstock participants recognized that they are just one portion of the overall recovery effort – the factors that originally brought about the decline have to be rectified before captive broodstocks can be effective, Berejikian replied. It depends on the individual circumstances, he said, but captive broodstocks can certainly play an important role. Powell noted that captive broodstocks have tended to be a last resort; the amount of starting material you begin with, genetically, is a major determinant of your ultimate success – there is a conundrum with respect to when to introduce broodstock technology to maximize your chances of success. I think anyone would rather use habitat improvements to bring about restoration, added Herb Pollard; in a case such as the Redfish Lake sockeye, where the migration corridor is the problem, that simply isn't feasible, and a captive broodstock program is the only way to keep that stock from blinking out.

Another participant noted that the Ne/N ratio in the sockeye program is very low, and asked the reason for that. A portion of those fish go to production, while another portion is retained for captive broodstock, Powell replied. We have to rely on the success of the fish in the captive program to maintain or improve that Ne/N ratio, he said.

***4. Session 2: Growth and Survival in Captivity.***

The moderator for this session was Tim Hoffnagel of ODFW, who introduced both the topic of this session and its first speaker.

***A. Infectuous Diseases in Fresh and Saltwater-Reared Captive Broodstock Programs***. Mark Strom of NMFS provided this presentation. At the close of his talk, one participant asked about infectuous salmon anemia (ISA) – can you forecast the likelihood of ISA reaching the West Coast given the prevalence of salmon ranching here? he asked. As long as no additional fish or eggs from the East Coast are moved to the West Coast, I wouldn't think there would be much risk, Strom replied. Do you have any realistic hope for an effective BKD vaccine down the road? Carlin McAuley asked. Yes, but it's going to take a lot of work to develop a better vaccine, Strom replied. I am hopeful, however, because of the results we're seeing.

***B. Efficacy and Toxicity of Erythromycin***. Bill Fairgrieve of the Pacific States Marine Fisheries Commission led the discussion on this topic. Afterward, one participant asked about egg survival – do you know that those eggs were alive after fertilization, or did you look only after shocking? We did look to make sure that they were eyed, Fairgrieve replied. However, the eggs could have been dead a day later, the participant noted. That's a valid point, Fairgrieve replied – the tradeoff when you're doing these programs is that you have to have enough spawners to do a valid analysis, and that can be a problem, he said. In response to another question, Fairgrieve noted that, when an epizooatic occurs, it's too late to feed; instead, injection is used. Erythromycin feeding is a prophylactic treatment only, he said.

***C. Captive Broodstock Nutrition: What Are We Forgetting***? Ann Gannam of the Fish and Wildlife Service led this presentation. When she concluded, one participant noted that for every nutrient that is deficient, broodstock conductors can expect to see a corresponding problem in juvenile development. It's a huge problem, and I'm not sure, given the financial realities of the feed business, who is going to solve it, Gannam agreed.

***D. The Influence of 70 Years of Wild, Freshwater Sequestration on Survival and Growth of a Normally-Anadromous Steelhead Stock in Freshwater Raceways, Marine Net-Pens, a Natural lake and At Liberty In the Ocean***. This presentation was provided by Frank Thrower of NMFS. At the conclusion of his remarks, one participant observed that everyone in the room is wondering about the survival results for the various crosses – one hybrid had very high survival, while the other hybrid had very low survival. What's the reason? he asked. We don't know, Thrower replied – they grew faster, their densities were slightly lower, so the following year, we split them out and reared them in three different densities and saw the same result. Obviously, he said, it must be a genetic

influence. We then attempted to re-create the same cross, and that cross performed about the same as the other crosses, just to make it even more confusing.

Those results are hard to explain, observed another participant – they look to me like an environmental effect. I don't disagree, Thrower replied. Were you also monitoring freshwater and marine smolt survival at the same time? another participant asked. No, not at the same time, Thrower replied. Would it be possible to make that comparison? the participant asked. Yes, we will be able to do that in future years, Thrower replied.

***E. Growth Regulation in Captive Broodstock Rearing of Salmon***. Walt Dickhoff of NMFS led this presentation. At the conclusion of his remarks, one participant said temperature influences both growth rate and conversion efficiency, as well as the things that are converted. Any thought about the role of temperature on reproductive events, making physiologically available certain dietary components? There hasn't been a lot of work on temperature on reproductive efficiency? Dickhoff replied. There hasn't been a large-scale study on the interaction of temperature and reproductive efficiency, Penny Swanson added; that is a study we would like to do, but it will require a large-scale effort. There are insulin-like growth factors that we know respond to changes in temperature, said Dickhoff. We know temperature manipulations can help us grow fish for meat, added Swanson, but we don't know much, as yet, about how to use temperature to increase reproductive success, other than the studies that target the effects of temperature on precocious maturation.

It would seem that the production of early-maturing males might not necessarily be a bad thing, said another participant; in some ways, that would make it easier, from an inbreeding standpoint, and because that is a component of the population that might otherwise be lost. It's a normal part of the salmonid life-cycle, Dickhoff agreed, although we have seen much higher rates of precocial maturation in many of our captive stocks than we see in the wild. That's the problem, he said; we've seen precocial rates of maturation of up to 50% in some of our chinook stocks, which is unnaturally high.

Are we doing something wrong, in terms of our approach to feed rate? Paul Kline asked. Should we re-think our feeding protocols, perhaps reducing feed rates at a certain point prior to maturation, then giving them a late burst of feed? There are clearly opportunities to explore different feed regimes, Dickhoff replied; there simply isn't a lot of definitive data available on that question, currently. Another participant noted that when striped bass encounter poor feeding conditions during the summer prior to maturation, they generally enjoy very poor reproductive success when they finally do spawn. Another participant noted that, while his chinook program feeds its fish right up to maturation and Bodega Bay stops feeding six months prior to maturation, both programs enjoy similar rates of maturation success, although his program enjoys somewhat lower rates of fecundity.

***5. Session 3: Maturation and Reproduction.***

Penny Swanson moderated this session, introducing the topic area as well as the first speaker.

***A. Early Sexual Maturation in 1+ Age Male Spring Chinook Salmon: Examination of the Roles of Size and Fatness***. Karl Shearer of NMFS described his work in this area. At the conclusion of his presentation, in response to a question, Shearer noted that it is likely to be very difficult to prevent early male maturation in captive broodstocks, particularly for programs that do not have access to chilled or well water. You didn't segregate males and females in this study? asked one participant. No, replied Shearer. The participant said they do segregate males and females in the Livingston program, feeding the males a starvation diet and the females a normal ration. That approach has worked in two of the three years, he said. In response to a question from Carlin McAuley, the participant said his program sexes its fish by taking a fin clip for genetic analysis. The technique isn't 100% accurate, he said, but it has been helpful to our program.

Have you seen any dominance effects in the smaller vs. larger fish in your program? another participant asked. In some of the studies where we grow a portion of the fish large, aggression appears to be a factor in the fish that grow larger, Shearer replied. Have you considered using identical studies with two different stocks of fish to get at the stock-specific effects of this approach? asked another participant. We have such a study ongoing right now, Shearer replied. Have you seen a different growth response with respect to length vs. weight? another participant asked. We have seen that the fish continue to grow in length, resulting in long, skinny fish, Shearer replied.

***B. Effects of Photoperiod, Temperature and GnRHa Treatment on the Timing of Spawning and Egg and Sperm Quality in Atlantic Salmon***. Erik Vikingstad of the University of Bergen led this presentation. When he finished, McAuley asked about the effects of GnRH on milt production; he noted that the data seemed to show production of up to 90 ml of milt. Actually, we saw yields of up to 130 ml from one implanted fish, Vikingstad replied. In response to another question, Vikingstad said the main thing with chilled water is that it seems to act in concert with photoperiod, which seems to be the most important factor in advancing or retarding spawn timing; he added that his program has seen a considerable increase in juvenile fish deformities if incubation temperatures exceed 10 degrees C. He noted that reducing rearing temperatures from 13 degrees C to 8 degrees C produced an advancement of up to two weeks in spawn timing. Were the males exposed to cold water as well as the females? another participant asked. Yes, Vikingstad replied. And you saw a response in the males as well as the females? the participant asked. Yes, Vikingstad replied.

***C. Got Milt? Lack of Adequate Milt Production in a Captive Broodstock Program for Redfish Lake Sockeye***. Carlin McAuley led this presentation. At its conclusion, one participant noted that the GnRH implants are timed release; typically, he said, if we use traditional implants too soon, the fish receive an initial blast of the

hormone, but too often, we see little or no response in terms of spawning success. Would there be any way to create an implant that would release a smaller amount of hormone, then spike up, then tail off? he asked. Yes, Swanson replied. Vikingstad noted that the microspheres his program uses produce a nice, steady dose over several weeks. In response to another question, McAuley said one problem with the sockeye program is that it is difficult to set up a spawning matrix that will accurately track male effect.

What proportion of your males do you implant, and what proportion do you allow to spawn naturally? Kline asked. We're implanting the males about two weeks ahead of when we think the females will be ovulating, McAuley replied, some time around the beginning of October; we generally try to implant about 30% of our available males.

Did you see higher milt volumes in your seawater-reared fish? another participant asked. Yes, although they were four-year-old fish, McAuley replied – in addition, it is generally easier to get the milt out of those fish. In the wild, how much of a role does social interaction play in encouraging final maturation, asked another participant, and if it has an influence, how can you mimic that in the hatchery? We've been looking at that question, replied Berejikian, but haven't seen any effect of hormones released into the environment in terms of bringing the later fish on. Basically, until the fish are ready, they don't do anything – once final maturation occurs, that's when you start to see the behavioral interactions, Berejikian said. McAuley added that the males and females are kept in the same pools, in the hopes that their proximity will encourage more synchronous spawn timing; so far, however, it seems to have little or no effect.

Another participant suggested that photoperiod should play a greater role in the timing of captive sockeye maturation. Another suggested that ovarian fluid is important to the fertilization environment; he said he has evidence that shows that removing it from the fertilization environment may negatively impact fertilization. I don't disagree, said Penny Swanson – there was a recent paper that showed that rinsing the eggs with ovarian fluid may enhance viability. Another suggested that putting the eggs into the bag with the milt, rather than pipetting the milt out of the bag and adding it to the eggs, would likely produce better results. We're open to anything, McAuley replied. Another suggested that McAuley's idea that exercise would benefit the captively-reared sockeye is a sound one, based on his experience. It's certainly one thing we've never done, McAuley agreed; in my opinion, given the long and arduous migratory route these fish are used to, it is worth trying.

## Day 2: June 26, 2002

### *5. Session 3 (Continued)*

***D. Use of Ultrasound to Determine Sex and Sexual Maturity in Spring Chinook Salmon Captive Broodstock.*** Marla Chaney of ODFW led this presentation. At its conclusion, McAuley asked about the Sacramento chinook program, and whether or not they are really able to sex their fish in December. Yes and no, was the reply; they go

through in December, but we're spawning in May and June, rather than August. Are the fish anaesthetized? asked another participant. We do anaesthetize them, but it is possible to do this procedure without anaesthesia, Chaney replied. In response to another question, Chaney said a typical ultrasound system costs between $12,000 and $15,000. Can the probe be mounted in a tube that you would just run the fish through? asked another participant. That is possible, but in our program, we use actual contact with the fish, Chaney replied.

***E. Factors Affecting Captive Salmonid Embryo Survival***. Jim Nagler of the University of Idaho led the discussion on this topic. Berejikian suggested that Nagler talk to Steve Schroeder about his work with wild and captive coho salmon in this area. That would be most interesting, Nagler replied. Is there a method to test the nutritional quality of captive vs. wild eggs? one participant asked. I'm sure there is, said Nagler. Swanson noted that much of the mortality on Nagler's study occurred prior to the two-cell stage – is that due to lack of fertilization? she asked. That's likely true, Nagler replied – we've seen considerable loss very early on. That's something we're just starting to look into, he said – it's uncertain where, exactly, it occurred.

***7. Session 4: Reintroduction Strategies***.

Berejikian moderated this session, providing a general introduction.

***A. Overview of Captive-Reared Salmon Reproductive Behavior***. Berejikian led this presentation. At the conclusion of his remarks, one participant noted that there may also be a need to consider which life-stage is collected; Berejikian agreed. It's certainly better to take eggs from a known number of redds, from a fish health and genetic standpoint, Berejikian replied. In response to another comment, Berejikian agreed that better cost-per-fish information would be useful.

***B. Spreading the Risk: Managing and Evaluating Reintroduction Strategies for Redfish Lake Sockeye Salmon***. Paul Kline led this presentation. When he concluded, one participant asked why, in his opinion, the net-pen releases are not doing as well as the fall direct releases of Redfish lake sockeye. That is counterintuitive, Kline agreed; however, those fish are reared at Eagle hatchery, and are reared more quickly to smolt size than the fish raised at Sawtooth for fall direct release. They are otherwise similar in body fat levels and rearing strategy. It is not a disease problem, he said. In response to another question, Kline said the sockeye release strategies are closely related to the carrying capacity of the systems to which those fish are being released. At this point, he said, we believe we can support 300-400 returning adults to the system, which is far lower than the targets in the NMFS delisting criteria, which are out of the question at this point. Another participant asked about hatchery adults vs. naturally-produced adults; Kline noted that only 10 of the 257 adults that returned in 2001 were the product of either eyed egg outplants or adult releases. We are doing a tremendous amount of pedigree tracking, he said, in an effort to reduce the loss of genetic material in this program. We want to utilize returning adults to the greatest extent possible in our spawning matrix, he said.

***C. Redd-to-Spawner Evaluation of Adult Atlantic Salmon***. Tim Sheehan of NMFS in Maine led this discussion on this topic. There were no questions following his presentation.

***D. Testing the Ability of Captively-Reared Chinook Salmon to Spawn Naturally***. David Venditti of IDFG provided this presentation. Following his remarks, one participant asked whether IDFG was able to track the success of peer crosses and of the wild/captive crosses. That is one of the things we're working on, Vendetti said; we take genetic samples from all of our captive fish, and will be taking similar samples from all of the outmigrating parr this spring. We have also taken clips from the wild carcasses we've been able to recover.

In response to another question, Venditti said the captive males were ready to spawn upon release, but were dominated by the wild males. Effectively, however, the result is the same, observed one participant -- the spawn timing of the captive males is delayed until the wild males disappear from the scene. That's correct, Venditti replied.

***E. Rearing and Reintroduction Strategies for Salmonid Captive Broodstocks: Implications for Olfactory Imprinting, Homing and Straying***. Andy Dittman of NMFS led this presentation. When he finished, no questions were offered.

***F. Pedigrees in Natural Populations Provide Important New Insight Into the Environmental and Genetic Correlates of Successful Supplementation***. This topic was presented by Paul Moran of NMFS. Following his remarks, no questions were offered.

***8. Overall Wrap-Up of Technical Sessions 1-4.***

One participant asked about the nutrient classification of Redfish Lake. Ultra-aligotrophic, Kline replied; we have initiated a regular series of nutrient applications to that system. Were the sockeye that returned in 2001 dominated genetically by one family? Penny Swanson asked. It was representative of what we produced, which was more than 60,000 juvenile sockeye, Kline replied. How do you explain the difference in adult return success between 2000 and 2001? another participant asked. Partly because we're one a three-year cycle of various release strategies; we emphasized smolt releases in the year that produced our 2000 returns, and presmolt releases in the year-class that produced the 2001 returns, Kline replied.

In response to another question, Venditti said the chinook adults released in the CSCPTOC program tend to disperse within a day after release; at total of 89 fish were released last year, 80 of which were observed by field personnel at some point.

***9. Session 5: Policy Perspectives on Captive Broodstocks.***

Pete Hassemer was the moderator for the last session of the day; he devoted a few minutes to a general introduction to this topic. Paul Kucera of the Nez Perce Tribe, Pat Patillo of WDFW and Herb Pollard were the presenters for this session.

***A. The Safety-Net Artificial Production Program (SNAPP)***. First up was Herb Pollard, who described SNAPP, NMFS' Safety-Net Artificial Propagation Program. In response to a question, Pollard said NMFS/BPA's July 16-17 presentation to the Council is to seek approval for SNAPP funding.

***B. WDFW's Captive Broodstock Planning Efforts and "Exit Strategy"***. Pat Patillo led this presentation. At the conclusion of Patillo's remarks, one participant asked whether he would consider the Redfish Lake sockeye program to be a success. I'm not extremely familiar with that program, Patillo replied, but I would have to say that, while there is no exit strategy for that program, it has to be considered a success because it has met its objective – the avoidance of extinction for that stock.

***C. Captive Broodstocks for the Recovery of Imperiled Salmonid Populations***. Paul Kucera led this presentation. At the conclusion of his remarks, one participant noted that Kucera's comment regarding whether or not managers should stand by and watch populations blink out reminds him of similar problems in medical care – it's an ethical question, to me. At what point do you compromise science in favor of ethics? he asked. Part of the kicker, from the tribal perspective, is that certain areas are crucially important, from a religious and cultural perspective. Those areas tend to be viewed as more crucial to the tribes, which can lead to some tradeoffs with other areas. Does your vision of restoring healthy populations include the removal of exotic species? another participant asked. We haven't been pursuing that management option aggressively, Kucera replied.

***D. Final Wrap-Up Discussion***. Hassemer provided a general summary of the main points expressed in the course of the policy session. The discussion returned to exit strategy; Hassemer reiterated that there is currently no exit strategy included in the Redfish Lake sockeye recovery project, because the factors that caused the precipitous decline of that stock, mainly the migration corridor, have yet to be addressed. Berejikian suggested one possible exit strategy: to continue the program for one generation past the point where the factors that caused the stock's decline have been addressed and rectified.

Another participant commented that many of those factors causing mortality are unlikely to be addressed in the foreseeable future; it may be that society has set the bar so high, in terms of delisting criteria, that those goals are not achievable, and maintaining certain esoteric stocks may be a luxury that society cannot afford. Another participant observed that the Redfish Lake program is often held up as a paragon of captive broodstock success, which has profoundly influenced the regard with which captive broodstock programs are held in the region; he said he was surprised to learn, in attending this workshop, that Redfish Lake sockeye are essentially a hatchery stock now, and even those adults that return are simply hatchery fish returning to the rack. Flagg took issue

with that characterization, noting that no one involved in the Redfish Lake sockeye program has ever characterized it as more than a stopgap measure to stave off extinction – no one has ever said we have restored that population, he said. Pollard added that, under the Endangered Species Act, federal agencies are required to work toward the recovery of listed species – we don't have the option of letting them go, he said.

Swanson observed that, while there are many questions about the cost and efficacy of captive broodstock programs, beyond their value as a recovery tool, there are much broader impacts – we're being driven to learn so much more about these animals, knowledge that will have broad implications in helping us manage these stocks.

I think we do have the common sense to know that these programs can't be sustained indefinitely, said Berejikian; we need to be aware of that fact, and enter into them cautiously in the future. Berejikian thanked everyone for coming and with that, the workshop was adjourned. Discussion summary prepared by Jeff Kuechle, BPA contractor.

## PART III. POSTER ABSTRACTS

*ABSTRACTS – DO NOT CITE*

### Growth History and Ovarian Development in Coho Salmon (*Oncorhynchus kisutch*)

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Several captive salmon broodstock programs have reported suboptimal gonadal development in maturing females. Research on iteroparous salmonids has shown that growth history has a pivotal role in controlling maturation. In the present study we manipulated growth in semelparous coho salmon during their adult seawater phase in order to determine critical periods where growth affects ovarian development.

During April 1999, yearling coho salmon from the Minter Creek Hatchery were PIT tagged and moved to seawater tanks. Fish were fed either a high (0.8% body weight/day) or low (0.5% body weight/day) ration through to June 2000. Further variations in growth histories were produced by redistributing fish between the two rations in November 1999 and February 2000. In June 2000, all fish were returned to high ration and fed up to spawning in November 2000, or terminal sampling of the remaining nonmature females in January 2001. Individual body weight and length were recorded every 2-3 months.

Ovary development in nonmaturing females was determined by histology. GSI and ovary histology of the nonmaturing females sampled in January 2001 showed two distinct categories; high GSI with healthy oocytes and small GSI with atretic oocytes. Growth histories were significantly different for females in these two categories of ovary development, and were also significantly different from the group of females that matured and spawned in November 2000. This provides evidence for the role of growth in female maturation as well as for the hypothesis that inappropriate growth history in semelparous salmon can result in functionally sterile females.

**Use of Cryopreserved Sperm in the Grande Ronde Sub-basin Spring Chinook Captive Broodstock Program**

Marla Chaney[1] and Mary Edwards[2]

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Successful cryopreservation of *Oncorhynchus tshawytscha* spermatozoa and the inclusion of these gametes in spawning matrices of the captive broodstock program are essential tools in preserving the genetic diversity of three listed spring chinook populations in the Grande Ronde Basin of Oregon. On site storage of gametes allows for their immediate incorporation when spawning protocols indicate 1) there are less than two appropriate fresh males available for any given matrix, and 2) the majority of fish to be spawned are of the same year class.

Fertilization success was initially measured by percent eye-up. Overall survival to eye-up in 1998 for all gametes fertilized with fresh or cryopreserved sperm was extremely poor, approximately 57% and 1% (N=3) respectively. However, variables other than egg and sperm quality can adversely affect eye-up rates; beginning in 1999 fertilization experiments were conducted on a sub-sample of eggs (N=10) from each matrix, with fertilization success evaluated during the blastomere phase. Fertilization rates for cryopreserved males at the blastomere and eyed-egg stage in 1999 increased to 31% (N=25) and 27.5% (N=26), respectively. In 2000 fertilization rates further increased to 43% (N= 55) and 40% (N=59), respectively.

Fertilization rates for fresh sperm continue to out-perform cryopreserved sperm, however, the ability to cryopreserve and use thawed gametes successfully has improved each year. The contribution of previously cryopreserved males, their ability to maintain genetic diversity, and mitigate the potential for sibling crosses, are extremely valuable components of the Captive Brood program.

**Tucannon River Spring Chinook Captive Broodstock Program: An Overview**

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The Washington Department of Fish and Wildlife initiated a captive broodstock program for Tucannon River spring chinook in 1997. The goal of the captive broodstock program is for the short-term preservation and rebuilding of this critically depressed run of ESA listed spring chinook in the Tucannon River, with the hope that natural production will eventually sustain itself. The project goal is to rear captive salmon selected from the supplementation program to adults, spawn them, rear their progeny, and release approximately 150,000 smolts annually into the Tucannon River between 2003-2007. These smolt releases, in combination with the current hatchery supplementation program (goal = 132,000 smolts) and wild production, is expected to produce 600-700 returning adult spring chinook to the Tucannon River each year from 2005-2010. Fish from the captive brood program matured earlier than fish from the supplementation program, with captive males beginning to mature at Age 2 and captive females starting to mature at Age 3. Fecundity, fork length (cm), and mortality to eye-up were significantly different ($P<0.05$) compared with hatchery and wild fish spawned in the supplementation program. However, egg size was similar among all groups. Spawn timing of captive brood fish has been 1-2 weeks later than fish captured from the Tucannon River for the supplementation program.

**Embryonic Development of Landlocked Atlantic Salmon and Brook Trout**

D. Marancik and G.R. Danner

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Embryonic development of Pacific Salmonid fishes can be divided into thirty visually identifiable stages between fertilization and hatch. The ability of an aquaculturist to readily recognize these stages in landlocked Atlantic salmon (*Salmo salar sebago*) and Eastern brook trout (*Salvelinus fontinalis*) could prove to be useful when estimating percentage survival, estimated time to hatch, or for determining a time of death. Past research has been successful at mapping out the embryonic development of Pacific salmon and correlating this development with water temperature (Velsen, 1980). This article illustrates these specific stages of development in landlocked Atlantic salmon and Eastern brook trout development because they are important aquaculture species in the eastern United States. Their embryos are somewhat smaller than Pacific salmon's embryos making it more challenging to identify developmental stages. Although exact details of development may vary between landlocked salmon and brook trout, the stages illustrated provide a reasonable depiction of development for the smaller salmonids.

**Effectiveness of Whole Cell Vaccines Against Bacterial Kidney Disease**

Linda D. Rhodes, Lee W. Harrell, Stephen C. Corbett, and Mark S. Strom

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Bacterial Kidney Disease (BKD) is a major source of mortality for salmonid populations at all life stages. Prevention and treatment of BKD is difficult because it can be transmitted both horizontally by shed bacteria in the water and vertically by bacteria contained in the egg. Currently, the most widely used drug treatment is administration of erythromycin, which delays BKD-associated mortality. However, persistent antibiotic use presents the risk of developing erythromycin resistance in *Renibacterium salmoninarum*, the etiological agent of BKD. Furthermore, long-term prophylactic use of erythromycin in chinook salmon captive broodstock can also cause toxic neurological reactions as well as decreased reproductive fitness. As an alternative treatment strategy, we are investigating vaccination against BKD. We tested two whole cell vaccines for their efficacy against BKD in yearling chinook salmon in pathogen-free seawater: Renogen, a commercially available vaccine consisting of an Arthrobacter species; and MT239, an attenuated strain of *R. salmoninarum*. Intraperitoneal (IP) vaccination with Renogen or MT239 significantly increased survival time after acute IP challenge with a virulent *R. salmoninarum* strain. When chinook salmon already infected with *R. salmoninarum* were treated IP with a combination of Renogen and MT239, a significant reduction in mortality was observed. These results suggest that whole cell vaccines can have both prophylactic and therapeutic effects against BKD.

**The Use of Fish Culture in the Recovery of the Endangered Sacramento River Winter-Run Chinook Salmon**

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The numbers of winter-run chinook salmon (WCS) *Oncorhynchus tshawytscha* returning to California's Sacramento River declined from 117,800 in 1967 to less than 200 in 1994. Consequently WCS were listed as threatened in 1990 and later reclassified as endangered in 1994. In 1988, the USFWS, CDFG, and NMFS entered into a cooperative agreement to improve habitat conditions for and increase numbers of WCS. Included in this agreement was the development of a propagation program at the Coleman National Fish Hatchery (NFH) beginning in 1989. A captive broodstock program was initiated at the U.C. Davis Bodega Marine Laboratory (BML) in 1991 to provide a secure genetic reserve to avert extinction. From 1991 to 1995 juvenile releases ranged from 10,000 to 51,000.

In 1995 two concerns threatened the continuation of the propagation program. First, adult hatchery WCS were returning to Battle Creek (site of Coleman NFH) not the Sacramento River where the fish were intended to spawn. Second, there was evidence of possible hybridization with other runs in the hatchery. The USFWS placed a moratorium on the collection of wild WCS adults until a rearing site on the main stem of the Sacramento River could be found, and until WCS could be genetically distinguished from other salmon runs. Therefore, in 1996 and 1997, only captive broodstock adults were spawned for the propagation program.

Within 2 years the Livingston Stone NFH was constructed at the base of Shasta Dam to assure imprinting and return of adults to the main stem Sacramento River. Genetic issues were resolved through development of a suite of micro-satellite markers by researchers at BML for run discrimination. The propagation program was restarted in 1998. Adult contribution from juveniles produced in the WCS propagation and captive broodstock programs have been documented in the ocean fishery and escapement monitoring in the Sacramento River.

**Spawning Performance of Sea-cage Reared Atlantic Salmon Released as Adults into the St. Croix River, Maine**

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Broodstock for several endangered populations of Atlantic salmon were reared in sea-cages by private aquaculture from river-specific eggs provided by the USFWS. Target spawning escapement was achieved for the endangered populations, and surplus broodstock were stocked into the St.Croix River, an international boundary water with an extirpated salmon population. In year one 412 females (1 female/3000 m2 cells rearing habitat) and 338 males were released approximately two weeks prior to the onset of spawning. A total of 170 redds (0.4 redds/female) were documented. Redds appeared to be well constructed and in appropriate habitat. Mean one-run density of young-of-the-year (YOY) salmon at 14 sites was 0.32 YOY/100 m2 cells (range 0.0 -2.0) at the end of the first growing season. In year two 305 females and 213 males (6 unknown gender) were stocked. A single trial of helicopter redd counts was attempted in addition to rigorous canoe redd counts. Both the total number of redds observed (430) and redds/female (1.4) increased substantially relative to year one. A sample of 29 redds were partially excavated two to four weeks post-spawning to evaluate the occurrence and viability of eggs. A sample of 20 eggs from each redd was preserved and examined for developmental stage, and a second 20 egg sample was collected for genetic analysis. All redds sampled contained a high proportion of viable eggs. A sample of 12 redds are currently being trapped to evaluate over-winter survival and quantify fry production.

**Reproductive Physiology of Adult Spring Chinook Salmon: Comparison of Captive Broodstock with Returning Hatchery Fish**

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Captively reared Snake River spring chinook salmon generally spawn two to six weeks later than returning wild fish of the same stock. The underlying causes of this delayed maturation are not known, although it is thought to be due to inappropriate rearing conditions. The goal of this study was to determine at what stage the captively-reared fish are delayed by comparing the process of gonadal maturation in a hatchery stock of Snake River spring chinook salmon after their upstream migration to that of captively-reared fish. Adult spring chinook salmon that returned to the Rapid River (RR) hatchery were sampled monthly from May through early September. Captively-reared female adults of the Lemhi River (LR) stock were sampled during May, August, and at spawning in late September and early October. Reproductive hormones were measured by immunoassay and gonad stage was determined histologically. The LR females were 50% smaller in body size, had smaller eggs, and higher relative fecundity than the RR females. Pituitary gonadotropins showed a similar pattern of change in the two groups, but levels were 3-4 fold lower in the LR than the RR stock. However, sex steroid levels in August were 2-fold higher in LR females. From May to August, gonadosomatic indices (GSI) increased 4 and 6-fold in the LR and RR females, respectively. LR females had significantly lower GSI than RR females, and were in earlier stages of vitellogenesis in May. Considerable gastric distention, atresia, and asymmetry in size of the two ovaries were found in LR fish. Together these data suggest that the delay may occur during ovarian growth, prior to transfer to fresh water. Atresia in the ovaries of the LR females may not be related to the delay in spawning since LR fish with healthy ovaries also spawned later than RR females.

## Dungeness River Captive Brood Chinook Program

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A captive broodstock program was initiated as part of a cooperative rebuilding effort for chinook salmon (*Oncorhynchus tshawytscha*) in the Dungeness River, using both land based freshwater and offshore saltwater technology. The freshwater phase consisted of brood years 1992 through 1997. The saltwater phase used brood years 1993 through 1996. A small percent of brood year 1993 were raised in shore-based saltwater tanks.

The freshwater program, using 20-foot circular fiberglass rearing vessels, started with 9,257 tagged wild juveniles. This generated 6,747 adults that produced 11,953,433 eggs. There are 419 fish still remaining in the program awaiting maturation. Egg viability to the eyed egg stage has averaged 94% survival. Mortality from the eyed egg stage to fry ponding has averaged 3.56%.

The saltwater program, using 20x40 foot net-pens, started with 3,727 tagged wild juveniles. This generated a total of 924 adults that produced 954,700 eggs. Egg viability to the eyed-egg stage averaged 67%. Mortality from the eyed-egg stage to fry ponding averaged 9.55%.